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(57) Abstract

Enzymatic RNA molecules which cleave ICAM-1 mRNA, IL-5 mRNA, *rel A* mRNA, TNF- α mRNA, RSV mRNA or RSV genomic RNA, or CML associated mRNA, and use of these molecules for the treatment of pathological conditions related to those mRNA-levels; ribonucleosides or nucleotides modified in 2', 3' or 5', methods for their synthesis, purification and deprotection; vectors containing multiple enzymatic nucleic acids, optionally in chimeric form with tRNAs; method for introducing enzymatic nucleic acids into cells by forming a complex with a second nucleic acid, where the complex is capable of taking an R-loop base-paired structure; method for altering a mutant nucleic acid *in vivo* by hybridization with an oligonucleotide capable of activating dsRNA deaminase, comprising an enzymatic activity or a chemical mutagen. Further are disclosed trans-cleaving or -ligating hairpin ribozymes lacking a substrate RNA moiety, as well as hammerhead ribozymes having an interconnecting loop between base pairs in stem II.

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METHOD AND REAGENT FOR INHIBITING THE EXPRESSION
OF DISEASE RELATED GENES

Background of the Invention

This invention relates to reagents useful as inhibitors of gene expression relating to diseases such as inflammatory or autoimmune disorders, chronic myelogenous leukemia, or respiratory tract illness.

5

Summary of the Invention

The invention features novel enzymatic RNA molecules, or ribozymes, and methods for their use for inhibiting the expression of disease related genes, e.g., ICAM-1, IL-5, relA, TNF- α , p210 bcr-abl, and respiratory syncytial virus genes. Such ribozymes can be used in a method for
10 treatment of diseases caused by the expression of these genes in man and other animals, including other primates.

Ribozymes are RNA molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence specific manner. Such enzymatic RNA molecules can be
15 targeted to virtually any RNA transcript, and efficient cleavage has been achieved *in vitro*. Kim et al., 84 Proc. Natl. Acad. Sci. USA 8788, 1987; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acids Research 1371, 1989.

Six basic varieties of naturally-occurring enzymatic RNAs are known
20 presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. Table 1 summarizes some of the characteristics of these ribozymes.

Ribozymes act by first binding to a target RNA. Such binding occurs
25 through the target RNA binding portion of a ribozyme which is held in close proximity to an enzymatic portion of the RNA which acts to cleave the target RNA. Thus, the ribozyme first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a
30 target RNA will destroy its ability to direct synthesis of an encoded protein. After a ribozyme has bound and cleaved its RNA target it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the effective concentration of ribozyme necessary to effect a therapeutic treatment is lower than that of an antisense oligonucleotide. The advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding, but also on the mechanism by which the molecule inhibits the expression of the RNA to which it binds. That is, the inhibition is caused by cleavage of the RNA target and so specificity is defined as the ratio of the rate of cleavage of the targeted RNA over the rate of cleavage of non-targeted RNA. This cleavage mechanism is dependent upon factors additional to those involved in base pairing. Thus, it is thought that the specificity of action of a ribozyme is greater than that of antisense oligonucleotide binding the same RNA site. With their catalytic activity and increased site specificity, ribozymes represent more potent and safe therapeutic molecules than antisense oligonucleotides.

Thus, in a first aspect, this invention relates to ribozymes, or enzymatic RNA molecules, directed to cleave RNA species encoding ICAM-1, IL-5, relA, TNF- α , p210^{bcr-abl}, or RSV proteins. In particular, applicant describes the selection and function of ribozymes capable of cleaving these RNAs and their use to reduce levels of ICAM-1, IL-5, relA, TNF- α , p210 bor-abl or RSV proteins in various tissues to treat the diseases discussed herein. Such ribozymes are also useful for diagnostic uses.

Applicant indicates that these ribozymes are able to inhibit expression of ICAM-1, IL-5, rel A, TNF- α , p210^{bcr-abl}, or RSV genes and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave target ICAM-1, IL-5, rel A, TNF- α , p210^{bcr-abl}, or RSV encoding mRNAs may be readily designed and are within the invention.

These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the

cleavage of RNA. Upon binding, the ribozymes cleave the target encoding mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, a therapeutic effect may be observed.

By "gene" is meant to refer to either the protein coding regions of the cognate mRNA, or any regulatory regions in the RNA which regulate synthesis of the protein or stability of the mRNA; the term also refers to those regions of an mRNA which encode the ORF of a cognate polypeptide product, and the proviral genome.

By "enzymatic RNA molecule" it is meant an RNA molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave RNA in that target. That is, the enzymatic RNA molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. This complementarity functions to allow sufficient hybridization of the enzymatic RNA molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. By "equivalent" RNA to a virus is meant to include those naturally occurring viral encoded RNA molecules associated with viral caused diseases in various animals, including humans, cats, simians, and other primates. These viral or viral-encoded RNAs have similar structures and equivalent genes to each other.

By "complementarity" it is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example, Hoogsteen type) of base-paired interactions.

In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or *Neurospora* VS RNA. Examples of such hammerhead motifs are described by Rossi *et al.*, 1992, *Aids Research and Human Retroviruses*, 8,183, of hairpin motifs by Hampel and Tritz, 1989 *Biochemistry*, 28, 4929, EP 0360257 and Hampel *et al.*, 1990, *Nucleic Acids Res.* 18,299 and an example of the hepatitis delta virus motif is described by Perotta and Been, 1992 *Biochemistry*, 31 16 of the RNaseP motif by Guerrier-Takada *et al.*, 1983 *Cell*, 35 849,

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expressed in eukaryotic cells from the appropriate DNA or RNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO93/23569, and Sullivan et al., PCT WO94/02595, both hereby incorporated in their
5 totality by reference herein; Ohkawa, J., et al., 1992, Nucleic Acids Symp. Ser. 27, 15-6; Taira, K. et al., Nucleic Acids Res. 19, 5125-30; Ventura, M., et al., 1993, Nucleic Acids Res. 21, 3249-55, Chowrira et al., 1994 J. Biol. Chem. 269, 25856).

By "inhibit" is meant that the activity or level of ICAM-1, Rel A, IL-5,
10 TNF- α , p210^{bcr-abl} or RSV encoding mRNA is reduced below that observed in the absence of the ribozyme, and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

Such ribozymes are useful for the prevention of the diseases and
15 conditions discussed above, and any other diseases or conditions that are related to the level of ICAM-1, IL-5, Rel A, TNF- α , p210^{bcr-abl} or RSV protein or activity in a cell or tissue. By "related" is meant that the inhibition of ICAM-1, IL-5, Rel A, TNF- α , p210^{bcr-abl} or RSV mRNA translation, and thus reduction in the level of, ICAM-1, IL-5, Rel A, TNF- α , p210^{bcr-abl} or
20 RSV proteins will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues
25 through the use of a catheter, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables 2,3,6-9, 11, 13, 15-23, 27, 28, 31, 33, 34, 36 and 37.

Examples of such ribozymes are shown in Tables 4-8, 10, 12, 14-16,
30 19-22, 24, 26-28, 30, 32, 34 and 36-38. Examples of such ribozymes consist essentially of sequences defined in these Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may
35 be present which do not interfere with such cleavage.

Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in
5 the above identified Tables can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in the above identified Tables can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of
10 two base-paired stem structure can form. The sequence listed in the above identified Tables may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

In another aspect of the invention, ribozymes that cleave target
15 molecules and inhibit ICAM-1, IL-5, Rel A, TNF- α , p210^{bcr-abl} or RSV gene expression are expressed from transcription units inserted into DNA, RNA, or viral vectors. Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA or RNA expression vector. Transcription of the
20 ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers,
25 silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 *Proc. Natl. Acad. Sci. USA*, 87, 6743-7; Gao and Huang 1993 *Nucleic Acids Res.*, 21, 2867-72; Lieber et al., 1993 *Methods Enzymol.*, 217, 47-66; Zhou et al.,
30 1990 *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 *Antisense Res. Dev.*, 2, 3-15; Ojwang et al., 1992 *Proc. Natl. Acad. Sci. USA*, 90, 6340-4; L'Huillier et al., 1992 *EMBO J.* 11, 4411-8; Lisiewicz et al., 1993 *Proc. Natl. Acad. Sci. U.S.A.*, 90 8000-4). The above ribozyme transcription units can
35 be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors

(such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors).

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from
5 the claims.

Description Of The Preferred Embodiments

The drawings will first briefly be described.

Drawings:

Figure 1 is a diagrammatic representation of the hammerhead
10 ribozyme domain known in the art. Stem II can be ≥ 2 base-pair long.

Figure 2(a) is a diagrammatic representation of the hammerhead ribozyme domain known in the art; Figure 2(b) is a diagrammatic representation of the hammerhead ribozyme as divided by Uhlenbeck (1987, *Nature*, 327, 596-600) into a substrate and enzyme portion; Figure
15 2(c) is a similar diagram showing the hammerhead divided by Haseloff and Gerlach (1988, *Nature*, 334, 585-591) into two portions; and Figure 2(d) is a similar diagram showing the hammerhead divided by Jeffries and Symons (1989, *Nucl. Acids. Res.*, 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the general structure of a
20 hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, n is 1,2,3 or 4) and helix 5 can be optionally provided of length 2 or more bases (preferably 3-20 bases, *i.e.*, m is from 1-20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (*i.e.*, r is ≥ 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4-20
25 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is
30 preferred. Helix 1 and 4 can be of any size (*i.e.*, o and p is each independently from 0 to any number, *e.g.* 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be

modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, *i.e.*, without a connecting loop. The connecting loop when present may be a ribonucleotide with or without
5 modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases. "____" refers to a covalent bond.

Figure 4 is a representation of the general structure of the hepatitis
10 delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 is a diagrammatic representation of the genetic map of RSV strain A2.

15 Figure 7 is a diagrammatic representation of the solid-phase synthesis of RNA.

Figure 8 is a diagrammatic representation of exocyclic amino protecting groups for nucleic acid synthesis.

Figure 9 is a diagrammatic representation of the deprotection of RNA.

20 Figure 10 is a graphical representation of the cleavage of an RNA substrate by ribozymes synthesized, deprotected and purified using the improved methods described herein.

Figure 11 is a schematic representation of a two pot deprotection protocol. Base deprotection is carried out with aqueous methyl amine at 65
25 °C for 10 min. The sample is dried in a speed-vac for 2-24 hours depending on the scale of RNA synthesis. Silyl protecting group at the 2'-hydroxyl position is removed by treating the sample with 1.4 M anhydrous HF at 65°C for 1.5 hours.

Figure 12 is a schematic representation of a one pot deprotection of
30 RNA synthesized using RNA phosphoramidite chemistry. Anhydrous methyl amine is used to deprotect bases at 65°C for 15 min. The sample is allowed to cool for 10 min before adding TEA•3HF reagent, to the same

pot, to remove protecting groups at the 2'-hydroxyl position. The deprotection is carried out for 1.5 hours.

5 Figs. 13a - b is a HPLC profile of a 36 nt long ribozyme, targeted to site B. The RNA is deprotected using either the two pot or the one pot deprotection protocol. The peaks corresponding to full-length RNA is indicated. The sequence for site B is CCUGGGCCAGGGAUUA AUGGAGAUGCCCACU.

Figure 14 is a graph comparing RNA cleavage activity of ribozymes deprotected by two pot vs one pot deprotection protocols.

10 Figure 15 is a schematic representation of an improved method of synthesizing RNA containing phosphorothioate linkages.

Figure 16 shows RNA cleavage reaction catalyzed by ribozymes containing phosphorothioate linkages. Hammerhead ribozyme targeted to site C is synthesized such that 4 nts at the 5' end contain phosphorothioate linkages. P=O refers to ribozyme without phosphorothioate linkages. P=S refers to ribozyme with phosphorothioate linkages. The sequence for site C is UCAUUUUGGCCAUCUC UCCCUUCAGGCGUGG.

Figure 17 is a schematic representation of synthesis of 2'-N-phthalimido-nucleoside phosphoramidite.

20 Figure 18 is a diagrammatic representation of a prior art method for the solid-phase synthesis of RNA using silyl ethers, and the method of this invention using SEM as a 2'-protecting group.

Figure 19 is a diagrammatic representation of the synthesis of 2'-SEM-protected nucleosides and phosphoramidites useful for the synthesis of RNA. B is any nucleotide base as exemplified in the Figure, P is purine and I is inosine. Standard abbreviations are used throughout this application, well known to those in the art.

Figure 20 is a diagrammatic representation of a prior art method for deprotection of RNA using TBDMS protection of the 2'-hydroxyl group.

30 Figure 21 is a diagrammatic representation of the deprotection of RNA having SEM protection of the 2'-hydroxyl group.

Figure 22 is a representation of an HPLC chromatogram of a fully deprotected 10-mer of uridylic acid.

Figs. 23 - 25 are diagrammatic representations of hammerhead, hairpin or hepatitis delta virus ribozyme containing self-processing RNA transcript. Solid arrows indicate self-processing sites. Boxes indicate the sites of nucleotide substitution. Solid lines are drawn to show the binding sites of primers used in a primer-extension assay. Lower case letters indicate vector sequence present in the RNA when transcribed from a *HindIII*-linearized plasmid. (23) HH Cassette, transcript containing the hammerhead trans-acting ribozyme linked to a 3' cis-acting hammerhead ribozyme. The structure of the hammerhead ribozyme is based on phylogenetic and mutational analysis (reviewed by Symons, 1992 *supra*). The trans ribozyme domain extends from nucleotide 1 through 49. After 3'-end processing, the trans-ribozyme contains 2 non-ribozyme nucleotides (UC at positions 50 and 51) at its 3' end. The 3' processing ribozyme is comprised of nucleotides 44 through 96. Roman numerals I, II and III, indicate the three helices that contribute to the structure of the 3' cis-acting hammerhead ribozyme (Hertel et al., 1992 *Nucleic Acids Res.* 20, 3252). Substitution of G70 and A71 to U and G respectively, inactivates the hammerhead ribozyme (Ruffner et al., 1990 *Biochemistry* 29, 10695) and generates the HH(mutant) construct. (24) HP Cassette, transcript containing the hammerhead trans-acting ribozyme linked to a 3' cis-acting hairpin ribozyme. The structure of the hairpin ribozyme is based on phylogenetic and mutational analysis (Berzal-Herranz et al., 1993 *EMBO J.* 12, 2567). The trans-ribozyme domain extends from nucleotide 1 through 49. After 3'-end processing, the trans-ribozyme contains 5 non-ribozyme nucleotides (UGGCA at positions 50 to 54) at its 3' end. The 3' cis-acting ribozyme is comprised of nucleotides 50 through 115. The transcript named HP(GU) was constructed with a potential wobble base pair between G52 and U77; HP(GC) has a Watson-Crick base pair between G52 and C77. A shortened helix 1 (5 base pairs) and a stable tetraloop (GAAA) at the end of helix 1 was used to connect the substrate with the catalytic domain of the hairpin ribozyme (Feldstein & Bruening, 1993 *Nucleic Acids Res.* 21, 1991; Altschuler et al., 1992 *supra*). (25) HDV Cassette, transcript containing the trans-acting hammerhead ribozyme linked to a 3' cis-acting hepatitis delta virus (HDV) ribozyme. The secondary structure of the HDV ribozyme is as proposed by Been and

coworkers (Been et al., 1992 Biochemistry 31, 11843). The trans-ribozyme domain extends from nucleotides 1 through 48. After 3'-end processing, the trans-ribozyme contains 2 non-ribozyme nucleotides (AA at positions 49 to 50) at its 3' end. The 3' cis-acting HDV ribozyme is comprised of nucleotides 50 through 114. Roman numerals I, II, III & IV, indicate the location of four helices within the 3' cis-acting HDV ribozyme (Perrota & Been, 1991 Nature 350, 434). The Δ HDV transcript contains a 31 nucleotide deletion in the HDV portion of the transcript (nucleotides 84 through 115 deleted).

Fig. 26 is a schematic representation of a plasmid containing the insert encoding self-processing cassette. The figure is not drawn to scale.

Fig. 27 demonstrates the effect of 3' flanking sequences on RNA self-processing *in vitro*. H, Plasmid templates linearized with *Hind*III restriction enzyme. Transcripts from H templates contain four non-ribozyme nucleotides at the 3' end. N, Plasmid templates linearized with *Nde*I restriction enzyme. Transcripts from N templates contain 220 non-ribozyme nucleotides at the 3' end. R, Plasmid templates linearized with *Rca*I restriction enzyme. Transcripts from R templates contain 450 non-ribozyme nucleotides at the 3' end.

Fig. 28 shows the effect of 3' flanking sequences on the trans-cleavage reaction catalyzed by a hammerhead ribozyme. A 622 nt internally-labeled RNA (<10 nM) was incubated with ribozyme (1000 nM) under single turn-over conditions (Herschlag and Cech, 1990 Biochemistry 29, 10159). HH+2, HH+37, and HH+52 are trans-acting ribozymes produced by transcription from the HH, Δ HDV, and HH(mutant) constructs, respectively, and that contain 2, 37 and 52 extra nucleotides on the 3' end. The plot of the fraction of uncleaved substrate versus time was fit to a double exponential curve using the KaleidaGraph graphing program (Synergy Software, Reading, PA). A double exponential curve fit was used because the data points did not fall on a single exponential curve, presumably due to varying conformers of ribozyme and/or substrate RNA.

Fig. 29 shows RNA self-processing in OST7-1 cells. *In vitro* lanes contain full-length, unprocessed transcripts that were added to cellular lysates prior to RNA extraction. These RNAs were either pre-incubated with $MgCl_2$ (+) or with DEPC-treated water (-) prior to being hybridized

with 5' end-labeled primers. Cellular lanes contain total cellular RNA from cells transfected with one of the four self-processing constructs. Cellular RNA are probed for ribozyme expression using a sequence specific primer-extension assay. Solid arrows indicate the location of primer extension
 5 bands corresponding to Full-Length RNA and 3' Cleavage Products.

Figs. 30,31 are diagrammatic representations of self-processing cassettes that will release trans-acting ribozymes with defined, stable stem-loop structures at the 5' and the 3' end following self-processing. 30, shows various permutations of a hammerhead self-processing cassette. 31,
 10 shows various permutations of a hairpin self-processing cassette.

Figs. 32a-b Schematic representation of RNA polymerase III promoter structure. Arrow indicates the transcription start site and the direction of coding region. A, B and C, refer to consensus A, B and C box promoter sequences. I, refers to intermediate cis-acting promoter sequence. PSE,
 15 refers to proximal sequence element. DSE, refers to distal sequence element. ATF, refers to activating transcription factor binding element. ?, refers to cis-acting sequence element that has not been fully characterized. EBER, Epstein-Barr-virus-encoded-RNA. TATA is a box well known in the art.

Figs. 33a-e Sequence of the primary tRNA^{met} and Δ 3-5 transcripts. The A and B box are internal promoter regions necessary for pol III transcription. Arrows indicate the sites of endogenous tRNA processing. The Δ 3-5 transcript is a truncated version of tRNA wherein the sequence 3' of B box has been deleted (Adeniyi-Jones et al., 1984 *supra*). This
 20 modification renders the Δ 3-5 RNA resistant to endogenous tRNA processing.

Figure 34. Schematic representation of RNA structural motifs inserted into the Δ 3-5 RNA. Δ 3-5/HHI- a hammerhead (HHI) ribozyme was cloned at the 3' region of Δ 3-5 RNA; S3- a stable stem-loop structure was
 30 incorporated at the 3' end of the Δ 3-5/HHI chimera; S5- stable stem-loop structures were incorporated at the 5' and the 3' ends of Δ 3-5/HHI ribozyme chimera; S35- sequence at the 3' end of the Δ 3-5/HHI ribozyme chimera was altered to enable duplex formation between the 5' end and a complementary 3' region of the same RNA; S35Plus- in addition to
 35 structural alterations of S35, sequences were altered to facilitate additional

duplex formation within the non-ribozyme sequence of the $\Delta 3$ -5/HHI chimera.

Figures 35 and 36. Northern analysis to quantitate ribozyme expression in T cell lines transduced with $\Delta 3$ -5 vectors. 35) $\Delta 3$ -5/HHI and its variants were cloned individually into the DC retroviral vector (Sullenger et al., 1990 *supra*). Northern analysis of ribozyme chimeras expressed in MT-2 cells was performed. Total RNA was isolated from cells (Chomczynski & Sacchi, 1987 *Analytical Biochemistry* 162, 156-159), and transduced with various constructs described in Fig. 34. Northern analysis was carried out using standard protocols (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). Nomenclature is same as in Figure 34. This assay measures the level of expression from the type 2 pol III promoter. 36) Expression of S35 constructs in MT2 cells. S35 (+ribozyme), S35 construct containing HHI ribozyme. S35 (-ribozyme), S35 construct containing no ribozyme.

Figure 37. Ribozyme activity in total RNA extracted from transduced MT-2 cells. Total RNA was isolated from cells transduced with $\Delta 3$ -5 constructs described in Figs. 35 and 36. In a standard ribozyme cleavage reaction, 5 μ g total RNA and trace amounts of 5' terminus-labeled ribozyme target RNA were denatured separately by heating to 90°C for 2 min in the presence of 50 mM Tris-HCl, pH 7.5 and 10 mM MgCl₂. RNAs were renatured by cooling the reaction mixture to 37°C for 10-15 min. Cleavage reaction was initiated by mixing the labeled substrate RNA and total cellular RNA at 37°C. The reaction was allowed to proceed for ~ 18h, following which the samples were resolved on a 20 % urea-polyacrylamide gel. Bands were visualized by autoradiography.

Figures 38 and 39. Ribozyme expression and activity levels in S35-transduced clonal CEM cell lines. 38) Northern analysis of S35-transduced clonal CEM cell lines. Standard curve was generated by spiking known concentrations of in vitro transcribed S5 RNA into total cellular RNA isolated from non-transduced CEM cells. Pool, contains RNA from pooled cells transduced with S35 construct. Pool (-G418 for 3 Mo), contains RNA from pooled cells that were initially selected for resistance to G418 and then grown in the absence of G418 for 3 months. Lanes A through N contain RNA from individual clones that were generated from the pooled cells transduced with S35 construct. tRNA_i^{met}, refers to the

endogenous tRNA. S35, refers to the position of the ribozyme band. M, marker lane. 39) Activity levels in S35-transduced clonal CEM cell lines. RNA isolation and cleavage reactions were as described in Fig.37. Nomenclature is same as in Figs. 35 and 36 except, S, 5' terminus-labeled substrate RNA. P, 8 nt 5' terminus-labeled ribozyme-mediated RNA cleavage product.

Figures 40 and 41 are proposed secondary structures of S35 and S35 containing a desired RNA (HHI), respectively. The position of HHI ribozyme is indicated in figure 41. Intramolecular stem refers to the stem structure formed due to an intramolecular base-paired interaction between the 3' sequence and the complementary 5' terminus. The length of the stem ranges from 15-16 base-pairs. Location of the A and the B boxes are shown.

Figures 42 and 43 are proposed secondary structures of S35 plus and S35 plus containing HHI ribozyme.

Figures 44, 45, 46 and 47 are the nucleotide base sequences of S35, HHIS35, S35 Plus, and HHIS35 Plus respectively.

Figs. 48a-b is a general formula for pol III RNA of this invention.

Figure 49 is a diagrammatic representation of 5T construct. In this construct the desired RNA is located 3' of the intramolecular stem.

Figures 50 and 51 contain proposed secondary structures of 5T construct alone and 5T construct containing a desired RNA (HHI ribozyme) respectively.

Figure 52 is a diagrammatic representation of TRZ-tRNA chimeras. The site of desired RNA insertion is indicated.

Figure 53 shows the general structure of HHITRZ-A ribozyme chimera. A hammerhead ribozyme targeted to site I is inserted into the stem II region of TRZ-tRNA chimera.

Figure 54 shows the general structure of HPITRZ-A ribozyme chimera. A hairpin ribozyme targeted to site I is cloned into the indicated region of TRZ-tRNA chimera.

Figure 55 shows a comparison of RNA cleavage activity of HHITRZ-A, HHITRZ-B and a chemically synthesized HH hammerhead ribozymes.

Figure 56 shows expression of ribozymes in T cell lines that are stably transduced with viral vectors. M, markers; lane 1, non-transduced CEM cells; lanes 2 and 3, MT2 and CEM cells transduced with retroviral vectors; lanes 4 and 5, MT2 and CEM cells transduced with AAV vectors.

Figs. 57a-b Schematic diagram of adeno-associated virus and adenoviruses vectors for ribozyme delivery. Both vectors utilize one or more ribozyme encoding transcription units (RZ) based on RNA polymerase II or RNA polymerase III promoters. A. Diagram of an AAV-based vector containing minimal AAV sequences comprising the inverted terminal repeats (ITR) at each end of the vector genome, an optional selectable marker (Neo) driven by an exogenous promoter (Pro), a ribozyme transcription unit, and sufficient additional sequences (stuffer) to maintain a vector length suitable for efficient packaging. B. Diagram of ribozyme expressing adenovirus vectors containing deletions of one or more wild type adenovirus coding regions (cross-hatched boxes marked as E1, pIX, E3, and E4), and insertion of the ribozyme transcription unit at any or several of those regions of deletions.

Fig. 58 is a graph showing the effect of arm length variation on the activity of ligated hammerhead (HH) ribozymes. Nomenclature 5/5, 6/6, 7/7, 8/8 and so on refers to the number of base-pairs being formed between the ribozyme and the target. For example, 5/8 means that the HH ribozyme forms 5 bp on the 5' side and 8 bp on the 3' side of the cleavage site for a total of 13 bp. $-\Delta G$ refers to the free energy of binding calculated for base-paired interactions between the ribozyme and the substrate RNA (Turner and Sugimoto, 1988 *Ann. Rev. Biophys. Chem.* 17, 167). RPI A is a HH ribozyme with 6/6 binding arms.

Figs. 59 and 60 and 61 show cleavage of long substrate (622 nt) by ligated HH ribozymes.

Fig. 62 is a diagrammatic representation of a hammerhead ribozyme (HH-H) targeted against a site termed H. Variants of HH-H are also shown that contain either a 2 base-paired stem II (HH-H1 and HH-H2) or a 3 base-paired stem II (HH-H3 and HH-H4).

Figs. 63 and 64 show RNA cleavage activity of HH-I and its variants (see Fig.62). 63) cleavage of matched substrate RNA (15 nt). 64) cleavage of long substrate RNA (613 nt).

5 Figs. 65a-b is a schematic representation of a method of this invention to synthesize a full length hairpin ribozyme. No splint strand is required for ligation but rather the two fragments hybridize together at helix 4 prior to ligation. The only prerequisite is that the 3' fragment is phosphorylated at its 5' end and that the 3' end of the 5' fragment have a hydroxyl group. The hairpin ribozyme is targeted against site J. H1 and H2 are intermolecular
10 helices formed between the ribozyme and the substrate. H3 and H4 are intramolecular helices formed within the hairpin ribozyme motif. Arrow indicates the cleavage site.

Fig. 66 shows RNA cleavage activity of ligated hairpin ribozymes targeted against site J.

15 Figs. 67a-b is a diagrammatic representation of a Site K Hairpin Ribozyme (HP-K) showing the proposed secondary structure of the hairpin ribozyme-substrate complex as described in the art (Berzal-Herranz *et al.*, 1993 *EMBO. J.*12, 2567). The ribozyme has been assembled from two fragments (bimolecular ribozyme; Chowrira and Burke, 1992 *Nucleic Acids*
20 *Res.* 20, 2835); #H1 and H2 represent intermolecular helix formation between the ribozyme and the substrate. H3 and H4 represent intramolecular helix formation within the ribozyme (intermolecular helix in the case of bimolecular ribozyme). Left panel (HP-K1) indicates 4 base-paired helix 2 and the right panel (HP-K2) indicates 6 base-paired helix 2.
25 Arrow indicates the site of RNA cleavage. All the ribozymes discussed herein were chemically synthesized by solid phase synthesis using RNA phosphoramidite chemistry, unless otherwise indicated. Those skilled in the art will recognize that these ribozymes could also be made transcriptionally *in vitro* and *in vivo*.

30 Figure 68 is a graph showing RNA cleavage by hairpin ribozymes targeted to site K. A plot of fraction of the target RNA uncleaved (fraction uncleaved) as a function of time is shown. HP-K2 (6 bp helix 2) cleaves a 422 target RNA to a greater extent than the HP-K1 (4 bp helix 2).

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 422 nt region (containing hairpin site A) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed in a standard transcription buffer in the presence of [α - 32 P]CTP (Chowrira & Burke, 1991 *supra*). The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed by chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (1 μ M) and internally labeled 422 nt substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Figs. 69a-b is the Site L Hairpin Ribozyme (HP-L) showing proposed secondary structure of the hairpin ribozyme-substrate complex. The ribozyme was assembled from two fragments as described above. The nomenclature is the same as above.

Figure 70 shows RNA cleavage by hairpin ribozymes targeted to site L. A plot of fraction of the target RNA uncleaved (fraction uncleaved) as a function of time is shown. HP-L2 (6 bp helix 2) cleaves a 2 KB target RNA to a greater extent than the HP-L1 (4 bp helix 2). To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 2 kb region (containing hairpin site L) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. The cleavage reactions were carried out as described above.

Figs. 71a-b shows a Site M Hairpin Ribozyme (HP-M) with the proposed secondary structure of the hairpin ribozyme-substrate complex. The ribozyme was assembled from two fragments as described above.

5 Figure 72 is a graph showing RNA cleavage by hairpin ribozymes targeted to site M. The ribozymes were tested at both 20°C and at 26°C. To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 1.9 KB region (containing hairpin site M) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Cleavage reactions were carried out as described
10 above except that 20°C and at 26°C temperatures were used.

Figs. 73a-d shows various structural modifications of the present invention. A) Hairpin ribozyme lacking helix 5. Nomenclature is same as described under figure 3. B) Hairpin ribozyme lacking helix 4 and helix 5. Helix 4 is replaced by a nucleotide loop wherein q is ≥ 2 bases.
15 Nomenclature is same as described under figure 3. C) Hairpin ribozyme lacking helix 5. Helix 4 loop is replaced by a linker 103"L", wherein L is a non-nucleotide linker molecule (Benseler *et al.*, 1993 *J. Am. Chem. Soc.* 115, 8483; Jennings *et al.*, WO 94/13688). Nomenclature is same as described under figure 3. D) Hairpin ribozyme lacking helix 4 and helix 5.
20 Helix 4 is replaced by non-nucleotide linker molecule "L" (Benseler *et al.*, 1993 *supra*; Jennings *et al.*, *supra*). Nomenclature is same as described under figure 3.

Figs. 74a-b shows Hairpin ribozymes containing nucleotide spacer region "s" at the indicated location, wherein s is ≥ 1 base. Hairpin
25 ribozymes containing spacer region, can be synthesized as one fragment or can be assembled from multiple fragments. Nomenclature is same as described under figure 3.

Figs. 75a-e shows the structures of the 5'-C-alkyl-modified nucleotides. R_1 is as defined above. R is OH, H, O-protecting group, NH, or
30 any group described by the publications discussed above, and those described below. B is as defined in the Figure or any other equivalent nucleotide base. CE is cyanoethyl, DMT is a standard blocking group. Other abbreviations are standard in the art.

Figure 76 is a diagrammatic representation of the synthesis of 5'-C-alkyl-D-allose nucleosides and their phosphoramidites.

Figure 77 is a diagrammatic representation of the synthesis of 5'-C-alkyl-L-talose nucleosides and their phosphoramidites.

- 5 Figure 78 is a diagrammatic representation of hammerhead ribozymes targeted to site O containing 5'-C-methyl-L-talo modifications at various positions.

Figure 79 shows RNA cleavage activity of HH-O ribozymes. Fraction of target RNA uncleaved as a function of time is shown.

- 10 Figure 80 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al. Nucleic Acids Res.* 1992, 20, 3252) showing specific substitutions.

- 15 Figs. 81a-j shows the structures of various 2'-alkyl modified nucleotides which exemplify those of this invention. R groups are alkyl groups, Z is a protecting group.

Figure 82 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine.

Figure 83 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene uridine.

- 20 Figure 84 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene cytidine.

Figure 85 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene adenosine.

- 25 Figure 86 is a diagrammatic representation of the synthesis of 2'-C-carboxymethylidene uridine, 2'-C-methoxycarboxymethylidene uridine and derivatized amidites thereof. X is CH₃ or alkyl as discussed above, or another substituent.

Figure 87 is a diagrammatic representation of a synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonates.

Figure 88 is a diagrammatic representation of the synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonate 3'-phosphoramidites, dimers and solid supported dimers.

Figure 89 is a diagrammatic representation of the synthesis of
5 nucleoside 5'-deoxy-5'-difluoromethylene triphosphates.

Figures 90 and 91 are diagrammatic representations of the synthesis of 3'-deoxy-3'-difluoromethylphosphonates and dimers.

Figure 92 is a schematic representation of synthesizing RNA phosphoramidite of a nucleotide containing a 2'-hydroxyl group
10 modification of the present invention.

Figs. 93a-b describes a method for deprotection of oligonucleotides containing a 2'-hydroxyl group modification of the present invention.

Figure 94 is a diagrammatic representation of a hammerhead ribozyme targeted to site N. Positions of 2'-hydroxyl group substitution is
15 indicated.

Figure 95 shows RNA cleavage activity of ribozymes containing a 2'-hydroxyl group modification of the present invention. All RNA, represents hammerhead ribozyme (HHN) with no 2'-hydroxyl group modifications. U7-ala, represents HHN ribozyme containing 2'-NH-alanine modification at the
20 U7 position. U4/U7-ala, represents HHA containing 2'-NH-alanine modifications at U4 and U7 positions. U4 lys, represents HHA containing 2'-NH-lysine modification at U4 position. U7 lys, represents HHA containing 2'-NH-lysine modification at U7 position. U4/U7-lys, represents HHN containing 2'-NH-lysine modification at U4 and U7 positions.

25 Figures 96 and 97 are schematic representations of synthesizing (solid-phase synthesis) 3' ends of RNA with modification of the present invention. B, refers to either a base, modified base or an H.

Figure 98 and 99 are schematic representations of synthesizing (solid-phase synthesis) 5' ends of RNA with modification of the present
30 invention. B, refers to either a base, modified base or an H.

Figures 100 and 101 are general schematic representations of the invention.

Fig. 102a-d is a schematic representation of a method of the invention.

Fig. 103 is a graph of the results of the experiment diagrammed in figure 104.

Figure 104 is a diagrammatic representation of a fusion mRNA used
5 in the experiment diagrammed in Fig. 102.

Figure 105 is a diagrammatic representation of a method for selection of useful ribozymes of this invention.

Figure 106 generally shows R-loop formation, and an R-loop complex. In addition, it indicates the location at which ligands can be
10 provided to target the R-loop complex to cells using at least three different procedures, such as ligand receptor interaction, lipid or calcium phosphate mediated delivery, or electroporation.

Figure 107 shows a method for use of self-processing ribozymes to generate therapeutic ribozymes of unit length. This method is essentially
15 described by Draper et al., PCT WO 93/23509.

Figure 108 shows a method of linking ligands like folate, carbohydrate or peptides to R-loop forming RNA.

Ribozymes of this invention block to some extent ICAM-1, IL-5, rel A, TNF- α , p210^{bcr-abl}, or RSV genes expression and can be used to treat
20 diseases or diagnose such diseases. Ribozymes will be delivered to cells in culture and to tissues in animal models. Ribozyme cleavage of ICAM-1, IL-5, rel A, TNF- α , p210^{bcr-abl}, or RSV mRNA in these systems may prevent or alleviate disease symptoms or conditions.

I. Target sites

25 Targets for useful ribozymes can be determined as disclosed in Draper et al PCT WO93/23509, Sullivan *et al.*, PCT WO94/02595 as well as by Draper et al., PCT/US94/13129 and hereby incorporated by reference herein in totality. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such
30 methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. Such ribozymes can also be

optimized and delivered as described therein. While specific examples to animal and human RNA are provided, those in the art will recognize that the equivalent human RNA targets described can be used as described below. Thus, the same target may be used, but binding arms suitable for
5 targeting human RNA sequences are present in the ribozyme. Such targets may also be selected as described below.

It must be established that the sites predicted by the computer-based RNA folding algorithm correspond to potential cleavage sites. Hammerhead or hairpin ribozymes are designed that could bind and are
10 individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci., USA, 86 7706-7710) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm
15 lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

mRNA is screened for accessible cleavage sites by the method described generally in Draper et al., PCT WO93/23569 hereby incorporated by reference herein. Briefly, DNA oligonucleotides
20 representing potential hammerhead or hairpin ribozyme cleavage sites are synthesized. A polymerase chain reaction is used to generate a substrate for T7 RNA polymerase transcription from cDNA clones. Labeled RNA transcripts are synthesized *in vitro* from DNA templates. The oligonucleotides and the labeled transcripts are annealed, RNaseH is
25 added and the mixtures are incubated for the designated times at 37°C. Reactions are stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved is determined by autoradiographic quantitation using a phosphor imaging system. From these data, hammerhead or hairpin ribozyme sites are chosen as the
30 most accessible.

Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used
35 follows the procedure for normal RNA synthesis as described in Usman et al., 1987 *J. Am. Chem. Soc.*, 109, 7845 and in Scaringe et al., 1990

Nucleic Acids Res., 18, 5433 and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, phosphoramidites at the 3'-end. The average stepwise coupling yields are >98%. Inactive ribozymes are synthesized by substituting a U for G₅ and a U for A₁₄ (numbering from Hertel et al., 1992 *Nucleic Acids Res.*, 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840). Ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbach, 1989, *Methods Enzymol.*, 180, 51). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992 *TIBS* 17,34). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography and are resuspended in water.

Example 1: ICAM-1

Ribozymes that cleave ICAM-1 mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders. ICAM-1 function can be blocked therapeutically using monoclonal antibodies. Ribozymes have the advantage of being generally immunologically inert, whereas significant neutralizing anti-IgG responses can be observed with some monoclonal antibody treatments.

The following is a brief description of the physiological role of ICAM-1. The discussion is not meant to be complete and is provided only for understanding of the invention that follows. This summary is not an admission that any of the work described below is prior art to the claimed invention.

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface protein whose expression is induced by inflammatory mediators. ICAM-1 is required for adhesion of leukocytes to endothelial cells and for several immunological functions including antigen presentation, immunoglobulin production and cytotoxic cell activity. Blocking ICAM-1 function prevents immune cell recognition and activity during transplant rejection and in animal models of rheumatoid arthritis, asthma and reperfusion injury.

- Cell-cell adhesion plays a pivotal role in inflammatory and immune responses (Springer et al., 1987 *Ann. Rev. Immunol.* 5, 223-252). Cell adhesion is required for leukocytes to bind to and migrate through vascular endothelial cells. In addition, cell-cell adhesion is required for antigen presentation to T cells, for B cell induction by T cells, as well as for the cytotoxicity activity of T cells, NK cells, monocytes or granulocytes. Intercellular adhesion molecule-1 (ICAM-1) is a 110 kilodalton member of the immunoglobulin superfamily that is involved in all of these cell-cell interactions (Simmons et al., 1988 *Nature (London)* 331, 624-627).
- 10 ICAM-1 is expressed on only a limited number of cells and at low levels in the absence of stimulation (Dustin et al., 1986 *J. Immunol.* 137, 245-254). Upon treatment with a number of inflammatory mediators (lipopolysaccharide, γ -interferon, tumor necrosis factor- α , or interleukin-1), a variety of cell types (endothelial, epithelial, fibroblastic and hematopoietic cells) in a variety of tissues express high levels of ICAM-1 on their surface (Springer et al. *supra*; Dustin et al., *supra*; and Rothlein et al., 1988 *J. Immunol.* 141, 1665-1669). Induction occurs via increased transcription of ICAM-1 mRNA (Simmons et al., *supra*). Elevated expression is detectable after 4 hours and peaks after 16 - 24 hours of induction.
- 20 ICAM-1 induction is critical for a number of inflammatory and immune responses. *In vitro*, antibodies to ICAM-1 block adhesion of leukocytes to cytokine-activated endothelial cells (Boyd, 1988 *Proc. Natl. Acad. Sci. USA* 85, 3095-3099; Dustin and Springer, 1988 *J. Cell Biol.* 107, 321-331). Thus, ICAM-1 expression may be required for the extravasation of immune cells to sites of inflammation. Antibodies to ICAM-1 also block T cell killing, mixed lymphocyte reactions, and T cell-mediated B cell differentiation, suggesting that ICAM-1 is required for these cognate cell interactions (Boyd et al., *supra*). The importance of ICAM-1 in antigen presentation is underscored by the inability of ICAM-1 defective murine B cell mutants to stimulate antigen-dependent T cell proliferation (Dang et al., 1990 *J. Immunol.* 144, 4082-4091). Conversely, murine L cells require transfection with human ICAM-1 in addition to HLA-DR in order to present antigen to human T cells (Altmann et al., 1989 *Nature (London)* 338, 512-514). In summary, evidence *in vitro* indicates that ICAM-1 is required for cell-cell interactions critical to inflammatory responses, cellular immune responses, and humoral antibody responses.
- 35

By engineering ribozyme motifs we have designed several ribozymes directed against ICAM-1 mRNA sequences. These have been synthesized with modifications that improve their nuclease resistance. These ribozymes cleave ICAM-1 target sequences *in vitro*.

5 The sequence of human, rat and mouse ICAM-1 mRNA can be screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and that contain potential hammerhead or hairpin ribozyme cleavage sites can be identified. These sites are shown in Tables 2, 3, and 6-9. (All sequences
10 are 5' to 3' in the tables) While rat, mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility.

15 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 4 - 8 and 10. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity and may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

20 The ribozymes will be tested for function *in vivo* by exogenous delivery to human umbilical vein endothelial cells (HUVEC). Ribozymes will be delivered by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA or RNA vectors described above. Cytokine-induced ICAM-1 expression will be
25 monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. ICAM-1 mRNA levels will be assessed by Northern, by RNase protection, by primer extension or by quantitative RT-PCR analysis. Ribozymes that block the induction of ICAM-1 protein and mRNA by more than 90% will be identified.

30 As disclosed by Sullivan et al., PCT WO94/02595, incorporated by reference herein, ribozymes and/or genes encoding them will be locally delivered to transplant tissue *ex vivo* in animal models. Expression of the ribozyme will be monitored by its ability to block *ex vivo* induction of ICAM-1 mRNA and protein. The effect of the anti-ICAM-1 ribozymes on graft
35 rejection will then be assessed. Similarly, ribozymes will be introduced

into joints of mice with collagen-induced arthritis or rabbits with *Streptococcal* cell wall-induced arthritis. Liposome delivery, cationic lipid delivery, or adeno-associated virus vector delivery can be used. One dose (or a few infrequent doses) of a stable anti-ICAM-1 ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate inflammatory and immune responses in these diseases.

Uses

ICAM-1 plays a central role in immune cell recognition and function. Ribozyme inhibition of ICAM-1 expression can reduce transplant rejection and alleviate symptoms in patients with rheumatoid arthritis, asthma or other acute and chronic inflammatory disorders. We have engineered several ribozymes that cleave ICAM-1 mRNA. Ribozymes that efficiently inhibit ICAM-1 expression in cells can be readily found and their activity measured with regard to their ability to block transplant rejection and arthritis symptoms in animal models. These anti-ICAM-1 ribozymes represent a novel therapeutic for the treatment of immunological or inflammatory disorders.

The therapeutic utility of reduction of activity of ICAM-1 function is evident in the following disease targets. The noted references indicate the role of ICAM-1 and the therapeutic potential of ribozymes described herein. Thus, these targets can be therapeutically treated with agents that reduce ICAM-1 expression or function. These diseases and the studies that support a critical role for ICAM-1 in their pathology are listed below. This list is not meant to be complete and those in the art will recognize further conditions and diseases that can be effectively treated using ribozymes of the present invention.

- Transplant rejection

ICAM-1 is expressed on venules and capillaries of human cardiac biopsies with histological evidence of graft rejection (Briscoe et al., 1991 *Transplantation* 51, 537-539).

Antibody to ICAM-1 blocks renal (Cosimi et al., 1990 *J. Immunol.* 144, 4604-4612) and cardiac (Flavin et al., 1991 *Transplant. Proc.* 23, 533-534) graft rejection in primates.

A Phase I clinical trial of a monoclonal anti-ICAM-1 antibody showed significant reduction in rejection and a significant increase in graft function in human kidney transplant patients (Haug, et al., 1993 *Transplantation* 55, 766-72).

- Rheumatoid arthritis

- 5 ICAM-1 overexpression is seen on synovial fibroblasts, endothelial cells, macrophages, and some lymphocytes (Chin et al., 1990 *Arthritis Rheum* 33, 1776-86; Koch et al., 1991 *Lab Invest* 64, 313-20).

Soluble ICAM-1 levels correlate with disease severity (Mason et al., 1993 *Arthritis Rheum* 36, 519-27).

- 10 Anti-ICAM antibody inhibits collagen-induced arthritis in mice (Kakimoto et al., 1992 *Cell Immunol* 142, 326-37).

Anti-ICAM antibody inhibits adjuvant-induced arthritis in rats (Iigo et al., 1991 *J Immunol* 147, 4167-71).

- Myocardial ischemia, stroke, and reperfusion injury

- 15 Anti-ICAM-1 antibody blocks adherence of neutrophils to anoxic endothelial cells (Yoshida et al., 1992 *Am J Physiol* 262, H1891-8).

Anti-ICAM-1 antibody reduces neurological damage in a rabbit model of cerebral stroke (Bowes et al., 1993 *Exp Neurol* 119, 215-9).

- 20 Anti-ICAM-1 antibody protects against reperfusion injury in a cat model of myocardial ischemia (Ma et al., 1992 *Circulation* 86, 937-46).

- Asthma

Antibody to ICAM-1 partially blocks eosinophil adhesion to endothelial cells and is overexpressed on inflamed airway endothelium and epithelium *in vivo* (Wegner et al., 1990 *Science* 247, 456-9).

- 25 In a primate model of asthma, anti-ICAM-1 antibody blocks airway eosinophilia (Wegner et al., *supra*) and prevents the resurgence of airway inflammation and hyper-responsiveness after dexamethasone treatment (Gundel et al., 1992 *Clin Exp Allergy* 22, 569-75).

- Psoriasis

Surface ICAM-1 and a clipped, soluble version of ICAM-1 is expressed in psoriatic lesions and expression correlates with inflammation (Kellner et al., 1991 *Br J Dermatol* 125, 211-6; Griffiths 1989 *J Am Acad Dermatol* 20, 617-29; Schopf et al., 1993 *Br J Dermatol* 128, 34-7).

- 5 Anti-ICAM antibody blocks keratinocyte antigen presentation to T cells (Nickoloff et al., 1993 *J Immunol* 150, 2148-59).

- Kawasaki disease

Surface ICAM-1 expression correlates with the disease and is reduced by effective immunoglobulin treatment (Leung, et al., 1989 *Lancet* 2, 1298-302).

- 10 Soluble ICAM levels are elevated in Kawasaki disease patients; particularly high levels are observed in patients with coronary artery lesions (Furukawa et al., 1992 *Arthritis Rheum* 35, 672-7; Tsuji, 1992 *Arerugi* 41, 1507-14).

- 15 Circulating LFA-1⁺ T cells are depleted (presumably due to ICAM-1 mediated extravasation) in Kawasaki disease patients (Furukawa et al., 1993 *Scand J Immunol* 37, 377-80).

Example 2: IL-5

- 20 Ribozymes that cleave IL-5 mRNA represent a novel therapeutic approach to inflammatory disorders like asthma. The invention features use of ribozymes to treat chronic asthma, e.g., by inhibiting the synthesis of IL-5 in lymphocytes and preventing the recruitment and activation of eosinophils.

- 25 A number of cytokines besides IL-5 may also be involved in the activation of inflammation in asthmatic patients, including platelet activating factor, IL-1, IL-3, IL-4, GM-CSF, TNF- α , gamma interferon, VCAM, ILAM-1, ELAM-1 and NF- κ B. In addition to these molecules, it is appreciated that any cellular receptors which mediate the activities of the cytokines are also good targets for intervention in inflammatory diseases. These targets include, but are not limited to, the IL-1R and TNF- α R on keratinocytes, epithelial and endothelial cells in airways. Recent data suggest that certain
- 30 neuropeptides may play a role in asthmatic symptoms. These peptides include substance P, neurokinin A and calcitonin-gene-related peptides. These target genes may have more general roles in inflammatory diseases, but are currently assumed to have a role only in asthma.

Ribozymes of this invention block to some extent IL-5 expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of asthma (Clutterbuck et al., 1989 supra; Garssen et al., 1991 Am. Rev. Respir. Dis. 144, 931-938; Larsen et al., 1992 J. Clin. Invest. 89, 747-752; Mauser et al., 1993 supra). Ribozyme cleavage of IL-5 mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

The sequence of human and mouse IL-5 mRNA were screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 11, 13, and 14, 15. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. (In Table 12, lower case letters indicate positions that are not conserved between the Human and the Mouse IL-5 sequences.)

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 12, 14 - 16. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem loop II sequence of hammerhead ribozymes listed in Tables 12 and 14 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 15 and 16 (5'-CACGUUGUG-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables 12, 14 - 16 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against IL-5 mRNA sequences. These ribozymes are synthesized

with modifications that improve their nuclease resistance. The ability of ribozymes to cleave IL-5 target sequences *in vitro* is evaluated.

The ribozymes will be tested for function *in vivo* by analyzing IL-5 expression levels. Ribozymes will be delivered to cells by incorporation
5 into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA or RNA vectors. IL-5 expression will be monitored by biological assays, ELISA, by indirect immunofluorescence, and/or by FACS analysis. IL-5 mRNA levels will be assessed by Northern analysis, RNase protection or primer extension analysis or quantitative RT-PCR.
10 Ribozymes that block the induction of IL-5 activity and/or IL-5 mRNA by more than 90% will be identified.

Uses

Interleukin 5 (IL-5), a cytokine produced by CD4+ T helper cells and mast cells, was originally termed B cell growth factor II (reviewed by
15 Takatsu et al., 1988 Immunol. Rev. 102, 107). It stimulates proliferation of activated B cells and induces production of IgM and IgA. IL-5 plays a major role in eosinophil function by promoting differentiation (Clutterbuck et al., 1989 Blood 73, 1504-12), vascular adhesion (Walsh et al., 1990 Immunology 71, 258-65) and *in vitro* survival of eosinophils (Lopez et al.,
20 1988 J. Exp. Med. 167, 219-24). This cytokine also enhances histamine release from basophils (Hirai et al., 1990 J. Exp. Med. 172, 1525-8). The following summaries of clinical results support the selection of IL-5 as a primary target for the treatment of asthma:

Several studies have shown a direct correlation between the number
25 of activated T cells and the number of eosinophils from asthmatic patients vs. normal patients (Oehling et al., 1992 J. Investig. Allergol. Clin. Immunol. 2, 295-9). Patients with either allergic asthma or intrinsic asthma were treated with corticosteroids. The bronchoalveolar lavage was monitored for eosinophils, activated T helper cells and recovery of pulmonary function
30 over a 28 to 30 day period. The number of eosinophils and activated T helper cells decreased progressively with subsequent improvement in pulmonary function compared to intrinsic asthma patients with no corticosteroid treatment.

Bronchoalveolar lavage cells were screened for production of
35 cytokines using *in situ* hybridization for mRNA. *In situ* hybridization signals

were detected for IL-2, IL-3, IL-4, IL-5 and GM-CSF. Upregulation of mRNA was observed for IL-4, IL-5 and GM-CSF (Robinson et al., 1993 J. Allergy Clin. Immunol. 92, 313-24). Another study showed that upregulation of IL-5 transcripts from allergen challenged vs. saline challenged asthmatic patients (Krishnaswamy et al., 1993 Am. J. Respir. Cell. Mol. Biol. 9, 279-86).

An 18 patient study was performed to determine a mechanism of action for corticosteroid improvement of asthma symptoms. Improvement was monitored by methacholine responsiveness. A correlation was observed between the methacholine responsiveness, a reduction in the number of eosinophils, a reduction in the number of cells expressing IL-4 and IL-5 mRNA and an increase in number of cells expressing interferon-gamma.

Bronchial biopsies from 15 patients were analyzed 24 hours after allergen challenge (Bentley et al., 1993 Am. J. Respir. Cell. Mol. Biol. 8, 35-42). Increased numbers of eosinophils and IL-2 receptor positive cells were found in the biopsies. No differences in the numbers of total leukocytes, T lymphocytes, elastase-positive neutrophils, macrophages or mast cell subtypes were observed. The number of cells expressing IL-5 and GM-CSF mRNA significantly increased.

In another patient study, the eosinophil phenotype was the same for asthmatic patients and normal individuals. However, eosinophils from asthmatic patients had greater leukotriene C4 producing capacity and migration capacity. There were elevated levels of IL-3, IL-5 and GM-CSF in the circulation of asthmatics but not in normal individuals (Bruijnzeel et al., 1992 Schweiz. Med. Wochenschr. 122, 298-301).

Efficacy of antibody to IL-5 was assessed in a guinea pig asthma model. The animals were challenged with ovalbumin and assayed for eosinophilia and the responsiveness to the bronchoconstriction substance P. A 30 mg/kg dose of antibody administered i.p. blocked ovalbumin-induced increased sensitivity to substance P and blocked increases in bronchoalveolar and lung tissue accumulation of eosinophils (Mauser et al., 1993 Am. Rev. Respir. Dis. 148, 1623-7). In a separate study guinea pigs challenged for eight days with ovalbumin were treated with monoclonal antibody to IL-5. Treatment produced a reduction in the

number of eosinophils in bronchoalveolar lavage. No reduction was observed for unchallenged guinea pigs and guinea pigs treated with a control antibody. Antibody treatment completely inhibited the development of hyperreactivity to histamine and arecoline after ovalbumin challenge
5 (van Oosterhout et al., 1993 Am. Rev. Respir. Dis. 147, 548-52)

Results obtained from human clinical analysis and animal studies indicate the role of activated T helper cells, cytokines and eosinophils in asthma. The role of IL-5 in eosinophil development and function makes IL-5 a good candidate for target selection. The antibody studies neutralized
10 IL-5 in the circulation thus preventing eosinophilia. Inhibition of the production of IL-5 will achieve the same goal.

Asthma – a prominent feature of asthma is the infiltration of eosinophils and deposition of toxic eosinophil proteins (e.g. major basic protein, eosinophil-derived neurotoxin) in the lung. A number of T-cell-
15 derived factors like IL-5 are responsible for the activation and maintenance of eosinophils (Kay, 1991 J. Allergy Clin. Immun. 87, 893). Inhibition of IL-5 expression in the lungs can decrease the activation of eosinophils and will help alleviate the symptoms of asthma.

Atopy – is characterized by the development of type I hypersensitive
20 reactions associated with exposure to certain environmental antigens. One of the common clinical manifestations of atopy is eosinophilia (accumulation of abnormally high levels of eosinophils in the blood). Antibodies against IL-5 have been shown to lower the levels of eosinophils in mice (Cook et al., 1993 in Immunopharmacol. Eosinophils ed. Smith and
25 Cook, pp. 193-216, Academic, London, UK)

Parasitic infection-related eosinophilia– infections with parasites like helminths, can lead to severe eosinophilia (Cook et al., 1993 supra). Animal models for eosinophilia suggest that infection of mice, for example, can lead to blood, peritoneal and/or tissue eosinophilia, all of
30 which seem to be lowered to varying degrees by antibodies directed against IL-5.

Pulmonary infiltration eosinophilia– is characterised by accumulation of high levels of eosinophils in pulmonary parenchyma (Gleich, 1990 J. Allergy Clin. Immunol. 85, 422).

L-Tryptophan-associated eosinophilia-myalgia syndrome (EMS)— The EMS disease is closely linked to the consumption of L-tryptophan, an essential aminoacid used to treat conditions like insomnia (for review see Varga et al., 1993 J Invest. Dermatol. 100, 97s). Pathologic and histologic studies have demonstrated high levels of eosinophils and mononuclear inflammatory cells in patients with EMS. It appears that IL-5 and transforming growth factor play a significant role in the development of EMS (Varga et al., 1993 supra) by activating eosinophils and other inflammatory cells.

Thus, ribozymes of the present invention that cleave IL-5 mRNA and thereby IL-5 activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits IL-5 function is described above; available cellular and activity assays are numerous, reproducible, and accurate. Animal models for IL-5 function and for each of the suggested disease targets exist (Cook et al., 1993 supra) and can be used to optimize activity.

Example 3: NF- κ B

Ribozymes that cleave *rel A* mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders. Inflammatory mediators such as lipopolysaccharide (LPS), interleukin-1 (IL-1) or tumor necrosis factor- α (TNF- α) act on cells by inducing transcription of a number of secondary mediators, including other cytokines and adhesion molecules. In many cases, this gene activation is known to be mediated by the transcriptional regulator, NF- κ B. One subunit of NF- κ B, the *rel A* gene product (termed RelA or p65) is implicated specifically in the induction of inflammatory responses. Ribozyme therapy, due to its exquisite specificity, is particularly well-suited to target intracellular factors that contribute to disease pathology. Thus, ribozymes that cleave mRNA encoded by *rel A* or TNF- α may represent novel therapeutics for the treatment of inflammatory and autoimmune disorders.

The nuclear DNA-binding activity, NF- κ B, was first identified as a factor that binds and activates the immunoglobulin κ light chain enhancer in B cells. NF- κ B now is known to activate transcription of a variety of other cellular genes (*e.g.*, cytokines, adhesion proteins, oncogenes and viral

proteins) in response to a variety of stimuli (e.g., phorbol esters, mitogens, cytokines and oxidative stress). In addition, molecular and biochemical characterization of NF- κ B has shown that the activity is due to a homodimer or heterodimer of a family of DNA binding subunits. Each subunit bears a stretch of 300 amino acids that is homologous to the oncogene, *v-rel*. The activity first described as NF- κ B is a heterodimer of p49 or p50 with p65. The p49 and p50 subunits of NF- κ B (encoded by the *nf- κ B2* or *nf- κ B1* genes, respectively) are generated from the precursors NF- κ B1 (p105) or NF- κ B2 (p100). The p65 subunit of NF- κ B (now termed Rel A) is encoded by the *rel A* locus.

The roles of each specific transcription-activating complex now are being elucidated in cells (N.D. Perkins, et al., 1992 Proc. Natl Acad. Sci USA 89, 1529-1533). For instance, the heterodimer of NF- κ B1 and Rel A (p50/p65) activates transcription of the promoter for the adhesion molecule, VCAM-1, while NF- κ B2/RelA heterodimers (p49/p65) actually inhibit transcription (H.B. Shu, et al., Mol. Cell. Biol. 13, 6283-6289 (1993)). Conversely, heterodimers of NF- κ B2/RelA (p49/p65) act with Tat-I to activate transcription of the HIV genome, while NF- κ B1/RelA (p50/p65) heterodimers have little effect (J. Liu, N.D. Perkins, R.M. Schmid, G.J. Nabel, J. Virol. 1992 66, 3883-3887). Similarly, blocking *rel A* gene expression with antisense oligonucleotides specifically blocks embryonic stem cell adhesion; blocking NF- κ B1 gene expression with antisense oligonucleotides had no effect on cellular adhesion (Narayanan et al., 1993 Mol. Cell. Biol. 13, 3802-3810). Thus, the promiscuous role initially assigned to NF- κ B in transcriptional activation (M.J. Lenardo, D. Baltimore, 1989 Cell 58, 227-229) represents the sum of the activities of the *rel* family of DNA-binding proteins. This conclusion is supported by recent transgenic "knock-out" mice of individual members of the *rel* family. Such "knock-outs" show few developmental defects, suggesting that essential transcriptional activation functions can be performed by more than one member of the *rel* family.

A number of specific inhibitors of NF- κ B function in cells exist, including treatment with phosphorothioate antisense oligonucleotide, treatment with double-stranded NF- κ B binding sites, and over expression of the natural inhibitor MAD-3 (an I κ B family member). These agents have

been used to show that NF- κ B is required for induction of a number of molecules involved in inflammation, as described below.

- NF- κ B is required for phorbol ester-mediated induction of IL-6 (I. Kitajima, et al., Science 258, 1792-5 (1992)) and IL-8 (Kunsch and Rosen, 1993 Mol. Cell. Biol. 13, 6137-46).

•NF- κ B is required for induction of the adhesion molecules ICAM-1 (Eck, et al., 1993 Mol. Cell. Biol. 13, 6530-6536), VCAM-1 (Shu et al., *supra*), and E-selectin (Read, et al., 1994 J. Exp. Med. 179, 503-512) on endothelial cells.

- 10 •NF- κ B is involved in the induction of the integrin subunit, CD18, and other adhesive properties of leukocytes (Eck et al., 1993 *supra*).

- The above studies suggest that NF- κ B is integrally involved in the induction of cytokines and adhesion molecules by inflammatory mediators. Two recent papers point to another connection between NF- κ B and inflammation: glucocorticoids may exert their anti-inflammatory effects by inhibiting NF- κ B. The glucocorticoid receptor and p65 both act at NF- κ B binding sites in the ICAM-1 promoter (van de Stolpe, et al., 1994 J. Biol. Chem. 269, 6185-6192). Glucocorticoid receptor inhibits NF- κ B-mediated induction of IL-6 (Ray and Prefontaine, 1994 Proc. Natl Acad. Sci USA 91, 752-756). Conversely, overexpression of p65 inhibits glucocorticoid induction of the mouse mammary tumor virus promoter. Finally, protein cross-linking and co-immunoprecipitation experiments demonstrated direct physical interaction between p65 and the glucocorticoid receptor (*Id.*).

- 25 Ribozymes of this invention block to some extent NF- κ B expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of restenosis, transplant rejection and rheumatoid arthritis. Ribozyme cleavage of *relA* mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

- 30 The sequence of human and mouse *relA* mRNA can be screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 17, 18 and 21-22. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and

ribozymes thereafter designed, the human targetted sequences are of most utility.

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 19 - 22. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity and may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against *rel A* mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave *rel A* target sequences *in vitro* is evaluated.

The ribozymes will be tested for function *in vivo* by analyzing cytokine-induced VCAM-1, ICAM-1, IL-6 and IL-8 expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA and RNA vectors. Cytokine-induced VCAM-1, ICAM-1, IL-6 and IL-8 expression will be monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. *Rel A* mRNA levels will be assessed by Northern analysis, RNase protection or primer extension analysis or quantitative RT-PCR. Activity of NF- κ B will be monitored by gel-retardation assays. Ribozymes that block the induction of NF- κ B activity and/or *rel A* mRNA by more than 50% will be identified.

RNA ribozymes and/or genes encoding them will be locally delivered to transplant tissue *ex vivo* in animal models. Expression of the ribozyme will be monitored by its ability to block *ex vivo* induction of VCAM-1, ICAM-1, IL-6 and IL-8 mRNA and protein. The effect of the anti-*rel A* ribozymes on graft rejection will then be assessed. Similarly, ribozymes will be introduced into joints of mice with collagen-induced arthritis or rabbits with *Streptococcal* cell wall-induced arthritis. Liposome delivery, cationic lipid delivery, or adeno-associated virus vector delivery can be used. One dose (or a few infrequent doses) of a stable anti-*rel A* ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate inflammatory and immune responses in these diseases.

Uses

A therapeutic agent that inhibits cytokine gene expression, inhibits adhesion molecule expression, and mimics the anti-inflammatory effects of glucocorticoids (without inducing steroid-responsive genes) is ideal for the treatment of inflammatory and autoimmune disorders. Disease targets for such a drug are numerous. Target indications and the delivery options each entails are summarized below. In all cases, because of the potential immunosuppressive properties of a ribozyme that cleaves *rel A* mRNA, uses are limited to local delivery, acute indications, or *ex vivo* treatment.

10 •Rheumatoid arthritis (RA).

Due to the chronic nature of RA, a gene therapy approach is logical. Delivery of a ribozyme to inflamed joints is mediated by adenovirus, retrovirus, or adeno-associated virus vectors. For instance, the appropriate adenovirus vector can be administered by direct injection into the synovium: high efficiency of gene transfer and expression for several months would be expected (B.J. Roessler, E.D. Allen, J.M. Wilson, J.W. Hartman, B. L. Davidson, J. Clin. Invest. 92, 1085-1092 (1993)). It is unlikely that the course of the disease could be reversed by the transient, local administration of an anti-inflammatory agent. Multiple administrations may be necessary. Retrovirus and adeno-associated virus vectors would lead to permanent gene transfer and expression in the joint. However, permanent expression of a potent anti-inflammatory agent may lead to local immune deficiency.

 •Restenosis.

25 Expression of NF- κ B in the vessel wall of pigs causes a narrowing of the luminal space due to excessive deposition of extracellular matrix components. This phenotype is similar to matrix deposition that occurs subsequent to coronary angioplasty. In addition, NF- κ B is required for the expression of the oncogene *c-myc* (F.A. La Rosa, J.W. Pierce, G.E. Sonenshein, Mol. Cell. Biol. 14, 1039-44 (1994)). Thus NF- κ B induces smooth muscle proliferation and the expression of excess matrix components: both processes are thought to contribute to reocclusion of vessels after coronary angioplasty.

 •Transplantation.

NF- κ B is required for the induction of adhesion molecules (Eck et al., *supra*, K. O'Brien, et al., J. Clin. Invest. 92, 945-951 (1993)) that function in immune recognition and inflammatory responses. At least two potential modes of treatment are possible. In the first, transplanted organs are treated *ex vivo* with ribozymes or ribozyme expression vectors. Transient inhibition of NF- κ B in the transplanted endothelium may be sufficient to prevent transplant-associated vasculitis and may significantly modulate graft rejection. In the second, donor B cells are treated *ex vivo* with ribozymes or ribozyme expression vectors. Recipients would receive the treatment prior to transplant. Treatment of a recipient with B cells that do not express T cell co-stimulatory molecules (such as ICAM-1, VCAM-1, and/or B7 or B7-2) can induce antigen-specific anergy. Tolerance to the donor's histocompatibility antigens could result; potentially, any donor could be used for any transplantation procedure.

15 •Asthma.

Granulocyte macrophage colony stimulating factor (GM-CSF) is thought to play a major role in recruitment of eosinophils and other inflammatory cells during the late phase reaction to asthmatic trauma. Again, blocking the local induction of GM-CSF and other inflammatory mediators is likely to reduce the persistent inflammation observed in chronic asthmatics. Aerosol delivery of ribozymes or adenovirus ribozyme expression vectors is a feasible treatment.

•Gene Therapy.

Immune responses limit the efficacy of many gene transfer techniques. Cells transfected with retrovirus vectors have short lifetimes in immune competent individuals. The length of expression of adenovirus vectors in terminally differentiated cells is longer in neonatal or immune-compromised animals. Insertion of a small ribozyme expression cassette that modulates inflammatory and immune responses into existing adenovirus or retrovirus constructs will greatly enhance their potential.

Thus, ribozymes of the present invention that cleave *rel A* mRNA and thereby NF- κ B activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits NF- κ B

function is described above; available cellular and activity assays are number, reproducible, and accurate. Animal models for NF- κ B function (Kitajima, et al., *supra*) and for each of the suggested disease targets exist and can be used to optimize activity.

5 Example 4: TNF- α

Ribozymes that cleave the specific sites in TNF- α mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders.

Tumor necrosis factor- α (TNF- α) is a protein, secreted by activated leukocytes, that is a potent mediator of inflammatory reactions. Injection of
10 TNF- α into experimental animals can simulate the symptoms of systemic and local inflammatory diseases such as septic shock or rheumatoid arthritis.

TNF- α was initially described as a factor secreted by activated macrophages which mediates the destruction of solid tumors in mice (Old,
15 1985 Science 230, 4225-4231). TNF- α subsequently was found to be identical to cachectin, an agent responsible for the weight loss and wasting syndrome associated with tumors and chronic infections (Beutler, et al., 1985 Nature 316, 552-554). The cDNA and the genomic locus for TNF- α have been cloned and found to be related to TNF- β (Shakhov et al., 1990
20 J. Exp. Med. 171, 35-47). Both TNF- α and TNF- β bind to the same receptors and have nearly identical biological activities. The two TNF receptors have been found on most cell types examined (Smith, et al., 1990 Science 248, 1019-1023). TNF- α secretion has been detected from monocytes/macrophages, CD4+ and CD8+ T-cells, B-cells, lymphokine
25 activated killer cells, neutrophils, astrocytes, endothelial cells, smooth muscle cells, as well as various non-hematopoietic tumor cell lines (for a review see Turetskaya et al., 1991 in Tumor Necrosis Factor. Structure, Function, and Mechanism of Action B. B. Aggarwal, J. Vilcek, Eds. Marcel Dekker, Inc., pp. 35-60). TNF- α is regulated transcriptionally and
30 translationally, and requires proteolytic processing at the plasma membrane in order to be secreted (Kriegler et al., 1988 Cell 53, 45-53). Once secreted, the serum half life of TNF- α is approximately 30 minutes. The tight regulation of TNF- α is important due to the extreme toxicity of this cytokine. Increasing evidence indicates that overproduction of TNF- α

during infections can lead to severe systemic toxicity and death (Tracey & Cerami, 1992 Am. J. Trop. Med. Hyg. 47, 2-7).

Antisense RNA and Hammerhead ribozymes have been used in an attempt to lower the expression level of TNF- α by targeting specified cleavage sites [Sioud et al., 1992 J. Mol. Biol. 223; 831; Sioud WO 94/10301; Kisich and co-workers, 1990 abstract (FASEB J. 4, A1860; 1991 slide presentation (J. Leukocyte Biol. sup. 2, 70); December, 1992 poster presentation at Anti-HIV Therapeutics Conference in San Diego, CA; and "Development of anti-TNF- α ribozymes for the control of TNF- α gene expression"- Kisich, Doctoral Dissertation, 1993 University of California, Davis] listing various TNF α targeted ribozymes.

Ribozymes of this invention block to some extent TNF- α expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of septic shock and rheumatoid arthritis. Ribozyme cleavage of TNF- α mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

The sequence of human and mouse TNF- α mRNA can be screened for accessible sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 23, 25, and 27 - 28. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. (In Table 24, lower case letters indicate positions that are not conserved between the human and the mouse TNF- α sequences.)

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 24, 26 - 28. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Tables 24 and 26 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any

sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 27 and 28 (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequences listed in Tables 24, 26 - 28 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables or AAV.

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves TNF- α RNA is inserted into a plasmid DNA vector or an adenovirus DNA viral vector or AAV or alpha virus or retrovirus vectors. Viral vectors have been used to transfer genes to the intact vasculature or to joints of live animals (Willard et al., 1992 Circulation, 86, 1-473.; Nabel et al., 1990 Science, 249, 1285-1288) and both vectors lead to transient gene expression. The adenovirus vector is delivered as recombinant adenoviral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant adenovirus particles are locally administered to the site of treatment, e.g., through the use of an injection catheter, stent or infusion pump or are directly added to cells or tissues *ex vivo*.

In another preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves TNF- α RNA is inserted into a retrovirus vector for sustained expression of ribozyme(s).

By engineering ribozyme motifs we have designed several ribozymes directed against TNF- α mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave TNF- α target sequences *in vitro* is evaluated.

The ribozymes will be tested for function in cells by analyzing bacterial lipopolysaccharide (LPS)-induced TNF- α expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA vectors. TNF- α expression will be monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. TNF- α mRNA levels will be assessed by Northern analysis, RNase protection, primer extension

analysis or quantitative RT-PCR. Ribozymes that block the induction of TNF- α activity and/or TNF- α mRNA by more than 90% will be identified.

RNA ribozymes and/or genes encoding them will be locally delivered to macrophages by intraperitoneal injection. After a period of ribozyme uptake, the peritoneal macrophages are harvested and induced *ex vivo* with LPS. The ribozymes that significantly reduce TNF- α secretion are selected. The TNF- α can also be induced after ribozyme treatment with fixed *Streptococcus* in the peritoneal cavity instead of *ex vivo*. In this fashion the ability of TNF- α ribozymes to block TNF- α secretion in a localized inflammatory response are evaluated. In addition, we will determine if the ribozymes can block an ongoing inflammatory response by delivering the TNF- α ribozymes after induction by the injection of fixed *Streptococcus*.

To examine the effect of anti-TNF- α ribozymes on systemic inflammation, the ribozymes are delivered by intravenous injection. The ability of the ribozymes to inhibit TNF- α secretion and lethal shock caused by systemic LPS administration are assessed. Similarly, TNF- α ribozymes can be introduced into the joints of mice with collagen-induced arthritis. Either free delivery, liposome delivery, cationic lipid delivery, adeno-associated virus vector delivery, adenovirus vector delivery, retrovirus vector delivery or plasmid vector delivery in these animal model experiments can be used to supply ribozymes. One dose (or a few infrequent doses) of a stable anti-TNF- α ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate tissue damage in these inflammatory diseases.

Macrophage isolation.

To produce responsive macrophages 1 ml of sterile fluid thioglycollate broth (Difco, Detroit, MI.) was injected i.p. into 6 week old female C57b1/6NCR mice 3 days before peritoneal lavage. Mice were maintained as specific pathogen free in autoclaved cages in a laminar flow hood and given sterilized water to minimize "spontaneous" activation of macrophages. The resulting peritoneal exudate cells (PEC) were obtained by lavage using Hanks balanced salt solution (HBSS) and were plated at 2.5×10^5 /well in 96 well plates (Costar, Cambridge, MA.) with Eagles minimal essential medium (EMEM) containing 10% heat inactivated fetal

bovine serum. After adhering for 2 hours the wells were washed to remove non-adherent cells. The resulting cultures were 97% macrophages as determined by morphology and staining for non-specific esterase.

Transfection of ribozymes into macrophages:

- 5 The ribozymes were diluted to 2X final concentration, mixed with an equal volume of 11nM lipofectamine (Life Technologies, Gaithersburg, MD.), and vortexed. 100 ml of lipid:ribozyme complex was then added directly to the cells, followed immediately by 10 ml fetal bovine serum. Three hours after ribozyme addition 100 ml of 1 mg/ml bacterial
- 10 lipopolysaccharide (LPS) was added to each well to stimulate TNF production.

Quantitation of TNF- α in mouse macrophages:

- Supernatants were sampled at 0, 2, 4, 8, and 24 hours post LPS stimulation and stored at -70°C. Quantitation of TNF- α was done by a
- 15 specific ELISA. ELISA plates were coated with rabbit anti-mouse TNF- α serum at 1:1000 dilution (Genzyme) followed by blocking with milk proteins and incubation with TNF- α containing supernatants. TNF- α was then detected using a murine TNF- α specific hamster monoclonal antibody (Genzyme). The ELISA was developed with goat anti-hamster IgG coupled
- 20 to alkaline phosphatase.

Assessment of reagent toxicity:

- Following ribozyme/lipid treatment of macrophages and harvesting of supernatants viability of the cells was assessed by incubation of the cells with 5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium
- 25 bromide (MTT). This compound is reduced by the mitochondrial dihydrogenases, the activity of which correlates well with cell viability. After 12 hours the absorbance of reduced MTT is measured at 585 nm.

Uses

- The association between TNF- α and bacterial sepsis, rheumatoid
- 30 arthritis, and autoimmune disease make TNF- α an attractive target for therapeutic intervention [Tracy & Cerami 1992 supra; Williams et al., 1992 Proc. Natl. Acad. Sci. USA 89, 9784-9788; Jacob, 1992 J. Autoimmun. 5 (Supp. A), 133-143].

Septic Shock

Septic shock is a complication of major surgery, bacterial infection, and polytrauma characterized by high fever, increased cardiac output, reduced blood pressure and a neutrophilic infiltrate into the lungs and other major organs. Current treatment options are limited to antibiotics to reduce the bacterial load and non-steroidal anti-inflammatories to reduce fever. Despite these treatments in the best intensive care settings, mortality from septic shock averages 50%, due primarily to multiple organ failure and disseminated vascular coagulation. Septic shock, with an incidence of 200,000 cases per year in the United States, is the major cause of death in intensive care units. In septic shock syndrome, tissue injury or bacterial products initiate massive immune activation, resulting in the secretion of pro-inflammatory cytokines which are not normally detected in the serum, such as TNF- α , interleukin-1 β (IL-1 β), γ -interferon (IFN- γ), interleukin-6 (IL-6), and interleukin-8 (IL-8). Other non-cytokine mediators such as leukotriene b₄, prostaglandin E₂, C3a and C3d also reach high levels (de Boer et al., 1992 Immunopharmacology 24, 135-148).

TNF- α is detected early in the course of septic shock in a large fraction of patients (de Boer et al., 1992 supra). In animal models, injection of TNF- α has been shown to induce shock-like symptoms similar to those induced by LPS injection (Beutler et al., 1985 Science 229, 869-871); in contrast, injection of IL-1 β , IL-6, or IL-8 does not induce shock. Injection of TNF- α also causes an elevation of IL-1 β , IL-6, IL-8, PgE₂, acute phase proteins, and TxA₂ in the serum of experimental animals (de Boer et al., 1992 supra). In animal models the lethal effects of LPS can be blocked by pre-administration of anti-TNF- α antibodies. The cumulative evidence indicates that TNF- α is a key player in the pathogenesis of septic shock, and therefore a good candidate for therapeutic intervention.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the joints leading to bone destruction and loss of joint function. At the cellular level, autoreactive T- lymphocytes and monocytes are typically present, and the synoviocytes often have altered morphology and immunostaining patterns. RA joints have been shown to contain elevated levels of TNF- α , IL-1 α and IL-1 β , IL-6, GM-CSF, and TGF-

β (Abney et al., 1991 Imm. Rev. 119, 105-123), some or all of which may contribute to the pathological course of the disease.

Cells cultured from RA joints spontaneously secrete all of the pro-inflammatory cytokines detected *in vivo*. Addition of antisera against TNF-α to these cultures has been shown to reduce IL-1α/β production by these cells to undetectable levels (Abney et al., 1991 Supra). Thus, TNF-α may directly induce the production of other cytokines in the RA joint. Addition of the anti-inflammatory cytokine, TGF-β, has no effect on cytokine secretion by RA cultures. Immunocytochemical studies of human RA surgical specimens clearly demonstrate the production of TNF-α, IL-1α/β, and IL-6 from macrophages near the cartilage/pannus junction when the pannus is invading and overgrowing the cartilage (Chu et al., 1992 Br. J. Rheumatology 31, 653-661). GM-CSF was shown to be produced mainly by vascular endothelium in these samples. Both TNF-α and TGF-β have been shown to be fibroblast growth factors, and may contribute to the accumulation of scar tissue in the RA joint. TNF-α has also been shown to increase osteoclast activity and bone resorption, and may have a role in the bone erosion commonly found in the RA joint (Cooper et al., 1992 Clin. Exp. Immunol. 89, 244-250).

Elimination of TNF-α from the rheumatic joint would be predicted to reduce overall inflammation by reducing induction of MHC class II, IL-1α/β, IL-6, and GM-CSF, and reducing T-cell activation. Osteoclast activity might also fall, reducing the rate of bone erosion at the joint. Finally, elimination of TNF-α would be expected to reduce accumulation of scar tissue within the joint by removal of a fibroblast growth factor.

Treatment with an anti-TNF-α antibody reduces joint swelling and the histological severity of collagen-induced arthritis in mice (Williams et al., 1992 Proc. Natl. Acad. Sci. USA 89, 9784-9788). In addition, a study of RA patients who have received i.v. infusions of anti-TNF-α monoclonal antibody reports a reduction in the number and severity of inflamed joints after treatment. The benefit of monoclonal antibody treatment in the long term may be limited by the expense and immunogenicity of the antibody.

Psoriasis

Psoriasis is an inflammatory disorder of the skin characterized by keratinocyte hyperproliferation and immune cell infiltrate (Kupper, 1990 J.

Clin. Invest. 86, 1783-1789). It is a fairly common condition, affecting 1.5-2.0% of the population. The disorder ranges in severity from mild, with small flaky patches of skin, to severe, involving inflammation of the entire epidermis. The cellular infiltrate of psoriasis includes T-lymphocytes, neutrophils, macrophages, and dermal dendrocytes. The majority of T-lymphocytes are activated CD4⁺ cells of the T_H-1 phenotype, although some CD8⁺ and CD4⁻/CD8⁻ are also present. B lymphocytes are typically not found in abundance in psoriatic plaques.

Numerous hypotheses have been offered as to the proximal cause of psoriasis including auto-antibodies and auto-reactive T-cells, overproduction of growth factors, and genetic predisposition. Although there is evidence to support the involvement of each of these factors in psoriasis, they are neither mutually exclusive nor are any of them necessary and sufficient for the pathogenesis of psoriasis (Reeves, 1991 Semin. Dermatol. 10, 217).

The role of cytokines in the pathogenesis of psoriasis has been investigated. Among those cytokines found to be abnormally expressed were TGF- α , IL-1 α , IL-1 β , IL-1ra, IL-6, IL-8, IFN- γ , and TNF- α . In addition to abnormal cytokine production, elevated expression of ICAM-1, ELAM-1, and VCAM has been observed (Reeves, 1991 supra). This cytokine profile is similar to that of normal wound healing, with the notable exception that cytokine levels subside upon healing. Keratinocytes themselves have recently been shown to be capable of secreting EGF, TGF- α , IL-6, and TNF- α , which could increase proliferation in an autocrine fashion (Oxholm et al., 1991 APMIS 99, 58-64).

Nickoloff et al., 1993 (J Dermatol Sci. 6, 127-33) have proposed the following model for the initiation and maintenance of the psoriatic plaque:

Tissue damage induces the wound healing response in the skin. Keratinocytes secrete IL-1 α , IL-1 β , IL-6, IL-8, TNF- α . These factors activate the endothelium of dermal capillaries, recruiting PMNs, macrophages, and T-cells into the wound site.

Dermal dendrocytes near the dermal/epidermal junction remain activated when they should return to a quiescent state, and subsequently secrete cytokines including TNF- α , IL-6, and IL-8. Cytokine expression, in

turn, maintains the activated state of the endothelium, allowing extravasation of additional immunocytes, and the activated state of the keratinocytes which secrete TGF- α and IL-8. Keratinocyte IL-8 recruits immunocytes from the dermis into the epidermis. During passage through
5 the dermis, T-cells encounter the activated dermal dendrocytes which efficiently activate the T_H-1 phenotype. The activated T-cells continue to migrate into the epidermis, where they are stimulated by keratinocyte-expressed ICAM-1 and MHC class II. IFN- γ secreted by the T-cells synergizes with the TNF- α from dermal dendrocytes to increase
10 keratinocyte proliferation and the levels of TGF- α , IL-8, and IL-6 production. IFN- γ also feeds back to the dermal dendrocyte, maintaining the activated phenotype and the inflammatory cycle.

Elevated serum titres of IL-6 increases synthesis of acute phase proteins including complement factors by the liver, and antibody production
15 by plasma cells. Increased complement and antibody levels increases the probability of autoimmune reactions.

Maintenance of the psoriatic plaque requires continued expression of all of these processes, but attractive points of therapeutic intervention are TNF- α expression by the dermal dendrocyte to maintain activated
20 endothelium and keratinocytes, and IFN- γ expression by T-cells to maintain activated dermal dendrocytes.

There are 3 million patients in the United States afflicted with psoriasis. The available treatments for psoriasis are corticosteroids. The most widely prescribed are TEMOVATE (clobetasol propionate), LIDEX
25 (fluocinonide), DIPROLENE (betamethasone propionate), PSORCON (diflorasone diacetate) and TRIAMCINOLONE formulated for topical application. The mechanism of action of corticosteroids is multifactorial. This is a palliative therapy because the underlying cause of the disease remains, and upon discontinuation of the treatment the disease returns.
30 Discontinuation of treatment is often prompted by the appearance of adverse effects such as atrophy, telangiectasias and purpura. Corticosteroids are not recommended for prolonged treatments or when treatment of large and/or inflamed areas is required. Alternative treatments include retinoids, such as etretinate, which has been approved for
35 treatment of severe, refractory psoriasis. Alternative retinoid-based treatments are in advanced clinical trials. Retinoids act by converting

keratinocytes to a differentiated state and restoration of normal skin development. Immunosuppressive drugs such as cyclosporine are also in the advanced stages of clinical trials. Due to the nonspecific mechanism of action of corticosteroids, retinoids and immunosuppressives, these treatments exhibit severe side effects and should not be used for extended periods of time unless the condition is life-threatening or disabling. There is a need for a less toxic, effective therapeutic agent in psoriatic patients.

HIV and AIDS

The human immunodeficiency virus (HIV) causes several fundamental changes in the human immune system from the time of infection until the development of full-blown acquired immunodeficiency syndrome (AIDS). These changes include a shift in the ratio of CD4+ to CD8+ T-cells, sustained elevation of IL-4 levels, episodic elevation of TNF- α and TNF- β levels, hypergammaglobulinemia, and lymphoma/leukemia (Rosenberg & Fauci, 1990 Immun. Today 11, 176; Weiss 1993 Science 260, 1273). Many patients experience a unique tumor, Kaposi's sarcoma and/or unusual opportunistic infections (e.g. *Pneumocystis carinii*, cytomegalovirus, herpesviruses, hepatitis viruses, papilloma viruses, and tuberculosis). The immunological dysfunction of individuals with AIDS suggests that some of the pathology may be due to cytokine dysregulation.

Levels of serum TNF- α and IL-6 are often found to be elevated in AIDS patients (Weiss, 1993 supra). In tissue culture, HIV infection of monocytes isolated from healthy individuals stimulates secretion of both TNF- α and IL-6. This response has been reproduced using purified gp120, the viral coat protein responsible for binding to CD-4 (Buonaguro et al., 1992 J. Virol. 66, 7159). It has also been demonstrated that the viral gene regulator, Tat, can directly induce TNF transcription. The ability of HIV to directly stimulate secretion of TNF- α and IL-6 may be an adaptive mechanism of the virus. TNF- α has been shown to upregulate transcription of the LTR of HIV, increasing the number of HIV-specific transcripts in infected cells. IL-6 enhances HIV production, but at a post-transcriptional level, apparently increasing the efficiency with which HIV transcripts are translated into protein. Thus, stimulation of TNF- α secretion by the HIV virus may promote infection of neighboring CD4+ cells both by enhancing virus production from latently infected cells and by driving replication of the virus in newly infected cells.

The role of TNF- α in HIV replication has been well established in tissue culture models of infection (Sher et al., 1992 Immun. Rev. 127, 183), suggesting that the mutual induction of HIV replication and TNF- α replication may create positive feedback *in vivo*. However, evidence for the presence of such positive feedback in infected patients is not abundant. TNF- α levels are found to be elevated in some, but not all patients tested. Children with AIDS who were given zidovudine had reduced levels of TNF- α compared to those not given zidovudine (Cremoni et al., 1993 AIDS 7, 128). This correlation lends support to the hypothesis that reduced viral replication is physiologically linked to TNF- α levels. Furthermore, recently it has been shown that the polyclonal B cell activation associated with HIV infection is due to membrane-bound TNF- α . Thus, levels of secreted TNF- α may not accurately reflect the contribution of this cytokine to AIDS pathogenesis.

Chronic elevation of TNF- α has been shown to result in cachexia (Tracey et al., 1992 Am. J. Trop. Med. Hyg. 47, 2-7), increased autoimmune disease (Jacob, 1992 supra), lethargy, and immune suppression in animal models (Aderka et al., 1992 Isr. J. Med. Sci. 28, 126-130). The cachexia associated with AIDS may be associated with chronically elevated TNF- α frequently observed in AIDS patients. Similarly, TNF- α can stimulate the proliferation of spindle cells isolated from Kaposi's sarcoma lesions of AIDS patients (Barillari et al., 1992 J. Immunol 149, 3727).

A therapeutic agent that inhibits cytokine gene expression, inhibits adhesion molecule expression, and mimics the anti-inflammatory effects of glucocorticoids (without inducing steroid-responsive genes) is ideal for the treatment of inflammatory and autoimmune disorders. Disease targets for such a drug are numerous. Target indications and the delivery options each entails are summarized below. In all cases, because of the potential immunosuppressive properties of a ribozyme that cleaves the specified sites in TNF- α mRNA, uses are limited to local delivery, acute indications, or *ex vivo* treatment.

- Septic shock.

Exogenous delivery of ribozymes to macrophages can be achieved by intraperitoneal or intravenous injections. Ribozymes will be delivered by incorporation into liposomes or by complexing with cationic lipids.

•Rheumatoid arthritis (RA).

5 Due to the chronic nature of RA, a gene therapy approach is logical. Delivery of a ribozyme to inflamed joints is mediated by adenovirus, retrovirus, or adeno-associated virus vectors. For instance, the appropriate adenovirus vector can be administered by direct injection into the synovium: high efficiency of gene transfer and expression for several
10 months would be expected (B.J. Roessler, E.D. Allen, J.M. Wilson, J.W. Hartman, B. L. Davidson, J. Clin. Invest. 92, 1085-1092 (1993)). It is unlikely that the course of the disease could be reversed by the transient, local administration of an anti-inflammatory agent. Multiple administrations may be necessary. Retrovirus and adeno-associated virus
15 vectors would lead to permanent gene transfer and expression in the joint. However, permanent expression of a potent anti-inflammatory agent may lead to local immune deficiency.

•Psoriasis

20 The psoriatic plaque is a particularly good candidate for ribozyme or vector delivery. The stratum comeum of the plaque is thinned, providing access to the proliferating keratinocytes. T-cells and dermal dendrocytes can be efficiently targeted by trans-epidermal diffusion .

Organ culture systems for biopsy specimens of psoriatic and normal skin are described in current literature (Nickoloff et al., 1993 Supra).
25 Primary human keratinocytes are easily obtained and will be grown into epidermal sheets in tissue culture. In addition to these tissue culture models, the flaky skin mouse develops psoriatic skin in response to UV light. This model would allow demonstration of animal efficacy for ribozyme treatments of psoriasis.

30 •Gene Therapy.

Immune responses limit the efficacy of many gene transfer techniques. Cells transfected with retrovirus vectors have short lifetimes in immune competent individuals. The length of expression of adenovirus

vectors in terminally differentiated cells is longer in neonatal or immune-compromised animals. Insertion of a small ribozyme expression cassette that modulates inflammatory and immune responses into existing adenovirus or retrovirus constructs will greatly enhance their potential.

- 5 Thus, ribozymes of the present invention that cleave TNF- α mRNA and thereby TNF- α activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits TNF- α function is described above; available cellular and activity assays
10 are number, reproducible, and accurate. Animal models for TNF- α function and for each of the suggested disease targets exist and can be used to optimize activity.

Example 5: p210^{bcr-abl}

- 15 Chronic myelogenous leukemia exhibits a characteristic disease course, presenting initially as a chronic granulocytic hyperplasia, and invariably evolving into an acute leukemia which is caused by the clonal expansion of a cell with a less differentiated phenotype (i.e., the blast crisis stage of the disease). CML is an unstable disease which ultimately progresses to a terminal stage which resembles acute leukemia. This
20 lethal disease affects approximately 16,000 patients a year. Chemotherapeutic agents such as hydroxyurea or busulfan can reduce the leukemic burden but do not impact the life expectancy of the patient (e.g., approximately 4 years). Consequently, CML patients are candidates for bone marrow transplantation (BMT) therapy. However, for those patients
25 which survive BMT, disease recurrence remains a major obstacle (Apperley et al., 1988 Br. J. Haematol. 69, 239).

- 30 The Philadelphia (Ph) chromosome which results from the translocation of the *abl* oncogene from chromosome 9 to the *bcr* gene on chromosome 22 is found in greater than 95% of CML patients and in 10-25% of all cases of acute lymphoblastic leukemia [(ALL); Fourth International Workshop on Chromosomes in Leukemia 1982, Cancer Genet. Cytogenet. 11, 316]. In virtually all Ph-positive CMLs and approximately 50% of the Ph-positive ALLs, the leukemic cells express *bcr-abl* fusion mRNAs in which exon 2 (b2-a2 junction) or exon 3 (b3-a2
35 junction) from the major breakpoint cluster region of the *bcr* gene is spliced

to exon 2 of the *abl* gene. Heisterkamp et al., 1985 Nature 315, 758; Shtivelman et al., 1987, Blood 69, 971). In the remaining cases of Ph-positive ALL, the first exon of the *bcr* gene is spliced to exon 2 of the *abl* gene (Hooberman et al., 1989 Proc. Nat. Acad. Sci. USA 86, 4259; 5 Heisterkamp et al., 1988 Nucleic Acids Res. 16, 10069).

The b3-a2 and b2-a2 fusion mRNAs encode 210 kd bcr-abl fusion proteins which exhibit oncogenic activity (Daley et al., 1990 Science 247, 824; Heisterkamp et al., 1990 Nature 344, 251). The importance of the bcr-abl fusion protein (p210^{bcr-abl}) in the evolution and maintenance of the 10 leukemic phenotype in human disease has been demonstrated using antisense oligonucleotide inhibition of p210^{bcr-abl} expression. These inhibitory molecules have been shown to inhibit the *in vitro* proliferation of leukemic cells in bone marrow from CML patients. Szczylik et al., 1991 Science 253, 562).

15 Reddy, U.S. Patent 5,246,921 (hereby incorporated by reference herein) describes use of ribozymes as therapeutic agents for leukemias, such as chronic myelogenous leukemia (CML) by targeting the specific junction region of *bcr-abl* fusion transcripts. It indicates causing cleavage by a ribozyme at or near the breakpoint of such a hybrid chromosome, 20 specifically it includes cleavage at the sequence GUX, where X is A, U or G. The one example presented is to cleave the sequence 5' AGC AG AGUU (cleavage site) CAA AAGCCCU-3'.

Scanlon WO 91/18625, WO 91/18624, and WO 91/18913 and Snyder et al., WO93/03141 and WO94/13793 describe a ribozyme effective 25 to cleave oncogenic variants of H-ras RNA. This ribozyme is said to inhibit H-ras expression in response to external stimuli.

The invention features use of ribozymes to inhibit the development or expression of a transformed phenotype in man and other animals by modulating expression of a gene that contributes to the expression of CML. 30 Cleavage of targeted mRNAs expressed in pre-neoplastic and transformed cells elicits inhibition of the transformed state.

The invention can be used to treat cancer or pre-neoplastic conditions. Two preferred administration protocols can be used, either *in vivo* administration to reduce the tumor burden, or *ex vivo* treatment to

eradicate transformed cells from tissues such as bone marrow prior to reimplantation.

This invention features an enzymatic RNA molecule (or ribozyme) which cleaves mRNA associated with development or maintenance of CML. The mRNA targets are present in the 425 nucleotides surrounding the fusion sites of the *bcr* and *abl* sequences in the b2-a2 and b3-a2 recombinant mRNAs. Other sequences in the 5' portion of the *bcr* mRNA or the 3' portion of the *abl* mRNA may also be targeted for ribozyme cleavage. Cleavage at any of these sites in the fusion mRNA molecules will result in inhibition of translation of the fusion protein in treated cells.

The invention provides a class of chemical cleaving agents which exhibit a high degree of specificity for the mRNA causative of CML. Such enzymatic RNA molecules can be delivered exogenously or endogenously to afflicted cells. In the preferred hammerhead motif the small size (less than 40 nucleotides, preferably between 32 and 36 nucleotides in length) of the molecule allows the cost of treatment to be reduced.

The smallest ribozyme delivered for any type of treatment reported to date (by Rossi et al., 1992 *supra*) is an *in vitro* transcript having a length of 142 nucleotides. Synthesis of ribozymes greater than 100 nucleotides in length is very difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. Delivery of ribozymes by expression vectors is primarily feasible using only *ex vivo* treatments. This limits the utility of this approach. In this invention, an alternative approach uses smaller ribozyme motifs and exogenous delivery. The simple structure of these molecules also increases the ability of the ribozyme to invade targeted regions of the mRNA structure. Thus, unlike the situation when the hammerhead structure is included within longer transcripts, there are no non-ribozyme flanking sequences to interfere with correct folding of the ribozyme structure, as well as complementary binding of the ribozyme to the mRNA target.

The enzymatic RNA molecules of this invention can be used to treat human CML or precancerous conditions. Affected animals can be treated at the time of cancer detection or in a prophylactic manner. This timing of treatment will reduce the number of affected cells and disable cellular

replication. This is possible because the ribozymes are designed to disable those structures required for successful cellular proliferation.

Ribozymes of this invention block to some extent p210^{bcr-abl} expression and can be used to treat disease or diagnose such disease.

- 5 Ribozymes will be delivered to cells in culture and to tissues in animal models of CML. Ribozyme cleavage of *bcr-abl* mRNA in these systems may prevent or alleviate disease symptoms or conditions.

- 10 The sequence of human *bcr-abl* mRNA can be screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and that contain potential hammerhead or hairpin ribozyme cleavage sites can be identified. These sites are shown in Table 29 (All sequences are 5' to 3' in the tables). The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

- 15 The sequences of the chemically synthesized ribozymes most useful in this study are shown in Table 30. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Table 30 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables 30 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the
25 ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against *bcr-abl* mRNA sequences. These have been synthesized with modifications that improve their nuclease resistance as described above. These ribozymes cleave *bcr-abl* target sequences *in vitro*.

- 30 The ribozymes are tested for function *in vivo* by exogenous delivery to cells expressing *bcr-abl*. Ribozymes are delivered by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA vectors. Expression of *bcr-abl* is monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. Levels of

bcr-abl mRNA are assessed by Northern analysis, RNase protection, by primer extension analysis or by quantitative RT-PCR techniques. Ribozymes that block the induction of p210^{*bcr-abl*} protein and mRNA by more than 20% are identified.

5 Example 6: RSV

This invention relates to the use of ribozymes as inhibitors of respiratory syncytial virus (RSV) production, and in particular, the inhibition of RSV replication.

RSV is a member of the virus family paramyxoviridae and is classified
10 under the genus *Pneumovirus* (for a review see McIntosh and Chanock, 1990 in Virology ed. B.N. Fields, pp. 1045, Raven Press Ltd. NY). The infectious virus particle is composed of a nucleocapsid enclosed within an envelope. The nucleocapsid is composed of a linear negative single-stranded non-segmented RNA associated with repeating subunits of
15 capsid proteins to form a compact structure and thereby protect the RNA from nuclease degradation. The entire nucleocapsid is enclosed by the envelope. The size of the virus particle ranges from 150 - 300 nm in diameter. The complete life cycle of RSV takes place in the cytoplasm of infected cells and the nucleocapsid never reaches the nuclear
20 compartment (Hall, 1990 in Principles and Practice of Infectious Diseases ed. Mandell et al., Churchill Livingstone, NY).

The RSV genome encodes ten viral proteins essential for viral production. RSV protein products include two structural glycoproteins (G and F) found in the envelope spikes, two matrix proteins [M and M2 (22K)]
25 found in the inner membrane, three proteins localized in the nucleocapsid (N, P and L), one protein that is present on the surface of the infected cell (SH), and two nonstructural proteins [NS1 (1C) and NS2 (1B)] found only in the infected cell. The mRNAs for the 10 RSV proteins have similar 5' and 3' ends. UV-inactivation studies suggest that a single promoter is used
30 with multiple transcription initiation sites (Barik et al., 1992 J. Virol. 66, 6813). The order of transcription corresponding to the protein assignment on the genomic RNA is 1C, 1B, N, P, M, SH, G, F, 22K and L genes (Huang et al., 1985 Virus Res. 2, 157) and transcript abundance corresponds to the order of gene assignment (for example the 1C and 1B mRNAs are
35 much more abundant than the L mRNA. Synthesis of viral message begins

immediately after RSV infection of cells and reaches a maximum at 14 hours post-infection (McIntosh and Chanock, *supra*).

There are two antigenic subgroups of RSV, A and B, which can circulate simultaneously in the community in varying proportions in different years (McIntosh and Chanock, *supra*). Subgroup A usually predominates. Within the two subgroups there are numerous strains. By the limited sequence analysis available it seems that homology at the nucleotide level is more complete within than between subgroups, although sequence divergence has been noted within subgroups as well. Antigenic determinates result primarily from both surface glycoproteins, F and G. For F, at least half of the neutralization epitopes have been stably maintained over a period of 30 years. For G however, A and B subgroups may be related antigenically by as little as a few percent. On the nucleotide level, however, the majority of the divergence in the coding region of G is found in the sequence for the extracellular domain (Johnson et al., 1987, *Proc. Natl. Acad. Sci. USA* 84, 5625).

Respiratory Syncytial Virus (RSV) is the major cause of lower respiratory tract illness during infancy and childhood (Hall, *supra*) and as such is associated with an estimated 90,000 hospitalizations and 4500 deaths in the United States alone (Update: respiratory syncytial virus activity - United States, 1993, *Mmwr Morb Mortal Wkly Rep*, 42, 971). Infection with RSV generally outranks all other microbial agents leading to both pneumonia and bronchitis. While primarily affecting children under two years of age, immunity is not complete and reinfection of older children and adults, especially hospital care givers (McIntosh and Chanock, *supra*), is not uncommon. Immunocompromised patients are severely affected and RSV infection is a major complication for patients undergoing bone marrow transplantation.

Uneventful RSV respiratory disease resembles a common cold and recovery is in 7 to 12 days. Initial symptoms (rhinorrhea, nasal congestion, slight fever, etc.) are followed in 1 to 3 days by lower respiratory tract signs of infection that include a cough and wheezing. In severe cases, these mild symptoms quickly progress to tachypnea, cyanosis, and listlessness and hospitalization is required. In infants with underlying cardiac or respiratory disease, the progression of symptoms is especially rapid and can lead to respiratory failure by the second or third day of illness. With

modern intensive care however, overall mortality is usually less than 5% of hospitalized patients (McIntosh and Chanock, *supra*).

At present, neither an efficient vaccine nor a specific antiviral agent is available. An immune response to the viral surface glycoproteins can provide resistance to RSV in a number of experimental animals, and a subunit vaccine has been shown to be effective for up to 6 months in children previously hospitalized with an RSV infection (Tristram *et al.*, 1993, J. Infect. Dis. 167, 191). An attenuated bovine RSV vaccine has also been shown to be effective in calves for a similar length of time (Kubota *et al.*, 1992 J. Vet. Med. Sci. 54, 957). Previously however, a formalin-inactivated RSV vaccine was implicated in greater frequency of severe disease in subsequent natural infections with RSV (Connors *et al.*, 1992 J. Virol. 66, 7444).

The current treatment for RSV infection requiring hospitalization is the use of aerosolized ribavirin, a guanosine analog [Antiviral Agents and Viral Diseases of Man, 3rd edition. 1990. (eds. G.J. Galasso, R.J. Whitley, and T.C. Merigan) Raven Press Ltd., NY.]. Ribavirin therapy is associated with a decrease in the severity of the symptoms, improved arterial oxygen and a decrease in the amount of viral shedding at the end of the treatment period. It is not certain, however, whether ribavirin therapy actually shortens the patients' hospital stay or diminishes the need for supportive therapies (McIntosh and Chanock, *supra*). The benefits of ribavirin therapy are especially clear for high risk infants, those with the most serious symptoms or for patients with underlying bronchopulmonary or cardiac disease. Inhibition of the viral polymerase complex is supported as the main mechanism for inhibition of RSV by ribavirin, since viral but not cellular polypeptide synthesis is inhibited by ribavirin in RSV-infected cells (Antiviral Agents and Viral Diseases of Man, 3rd edition. 1990. (eds. G.J. Galasso, R.J. Whitley, and T.C. Merigan) Raven Press Ltd., NY). Since ribavirin is at least partially effective against RSV infection when delivered by aerosolization, it can be assumed that the target cells are at or near the epithelial surface. In this regard, RSV antigen had not spread any deeper than the superficial layers of the respiratory epithelium in autopsy studies of fatal pneumonia (McIntosh and Chanock, *supra*).

Jennings *et al.*, WO 94/13688 indicates that targets for specific types of ribozymes include respiratory syncytical virus.

The invention features novel enzymatic RNA molecules, or ribozymes, and methods for their use for inhibiting production of respiratory syncytial virus (RSV). Such ribozymes can be used in a method for treatment of diseases caused by these related viruses in man and other animals. The invention also features cleavage of the genomic RNA and mRNA of these viruses by use of ribozymes. In particular, the ribozyme molecules described are targeted to the *NS1 (1C)*, *NS2 (1B)* and *N* viral genes. These genes are known in the art (for a review see McIntosh and Chanock, 1990 *supra*).

10 Ribozymes that cleave the specified sites in RSV mRNAs represent a novel therapeutic approach to respiratory disorders. Applicant indicates that ribozymes are able to inhibit the activity of RSV and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described
15 that other ribozymes that cleave these sites in RSV mRNAs encoding 1C, 1B and N proteins may be readily designed and are within the invention. Also, those of ordinary skill in the art, will find that it is clear from the examples described that ribozymes cleaving other mRNAs encoded by RSV (*P*, *M*, *SH*, *G*, *F*, *22K* and *L*) and the genomic RNA may be readily
20 designed and are within the invention.

In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables 31, 33, 35, 37 and 38. Examples of such ribozymes are shown in Tables 32, 34, 36-38. Examples of such ribozymes consist essentially of sequences defined in these
25 Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

30 Ribozymes of this invention block to some extent RSV production and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of respiratory disorders. Ribozyme cleavage of RSV encoded mRNAs or the genomic RNA in these systems may alleviate disease symptoms.

While all ten RSV encoded proteins (1C, 1B, N, P, M, SH, 22K, F, G, and L) are essential for viral life cycle and are all potential targets for ribozyme cleavage, certain proteins (mRNAs) are more favorable for ribozyme targeting than the others. For example RSV encoded proteins 1C, 1B, SH and 22K are not found in other members of the family paramyxoviridae and appear to be unique to RSV. In contrast the ectodomain of the G protein and the signal sequence of the F protein show significant sequence divergence at the nucleotide level among various RSV sub-groups (Johnson *et al.*, 1987 *supra*). RSV proteins 1C, 1B and N are highly conserved among various subtypes at both the nucleotide and amino acid levels. Also, 1C, 1B and N are the most abundant of all RSV proteins.

The sequence of human RSV mRNAs encoding 1C, 1B and N proteins are screened for accessible sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 31, 33, 34, 37 and 38 (All sequences are 5' to 3' in the tables.) The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman *et al.*, 1987 J. Am. Chem. Soc., 109, 7845-7854 and in Scaringe *et al.*, 1990 Nucleic Acids Res., 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for G₅ and a U for A₁₄ (numbering from Hertel *et al.*, 1992 Nucleic Acids Res., 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). Hairpin ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant

groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992 *TIBS* 17, 34). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography and are resuspended in water.

5 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 32, 34, 36, 37 and 38. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of
10 hammerhead ribozymes listed in Tables 32 and 34 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 37 and 38 (5'-CACGUUGUG-3') can be altered (substitution,
15 deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequences listed in Tables 32, 34, 36, 37 and 38 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

20 By engineering ribozyme motifs we have designed several ribozymes directed against RSV encoded mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave target sequences *in vitro* is evaluated.

25 Numerous, common cell lines can be infected with RSV for experimental purposes. These include *HeLa*, *Vero* and several primary epithelial cell lines. A cotton rat animal model of experimental human RSV infection is also available, and the bovine RSV is quite homologous to the human viruses. Rapid clinical diagnosis is through the use of kits designed for the immunofluorescence staining of RSV-infected cells or an ELISA
30 assay, both of which are adaptable for experimental study. RSV encoded mRNA levels will be assessed by Northern analysis, RNase protection, primer extension analysis or quantitative RT-PCR. Ribozymes that block the induction of RSV activity and/or 1C, 1B and N protein encoding mRNAs by more than 90% will be identified.

Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described by Draper et al., PCT WO93/23569. The details will not be repeated here, but include altering the length of the ribozyme binding arms or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 Nature 344, 565; Pieken et al., 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International Publication No. WO 91/03162, as well as Jennings et al., WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan, et al., PCT WO94/02595, incorporated by reference herein, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. The RNA/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or stent. Alternative routes of delivery include, but are not limited to, intravenous injection, intramuscular injection, subcutaneous injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan, et al., supra and Draper, et al., supra which have been incorporated by reference herein.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given

pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells

5 (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. U S A, 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet

10 et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. U S A, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. U S A, 90, 6340-4; L'Huillier et al., 1992 EMBO J., 11, 4411-8; Lisiewicz et al., 1993 Proc. Natl. Acad. Sci. U. S. A., 90, 8000-4). The above ribozyme transcription units can be

15 incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral, or alpha virus vectors).

In a preferred embodiment of the invention, a transcription unit

20 expressing a ribozyme that cleaves target RNA is inserted into a plasmid DNA vector, a retrovirus DNA viral vector, an adenovirus DNA viral vector or an adeno-associated virus vector or alpha virus vector. These and other vectors have been used to transfer genes to live animals (for a review see Friedman, 1989 Science 244, 1275-1281; Roemer and Friedman, 1992

25 Eur. J. Biochem., 208, 211-225) and leads to transient or stable gene expression. The vectors are delivered as recombinant viral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant virus particles are locally administered to the site of treatment, e.g., through the

30 use of a catheter, stent or infusion pump.

Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells. The close relationship between ribozyme activity and the structure of the target RNA

35 allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By

using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNA associated with ICAM-1, relA, TNF- α , p210, bcr-abl or RSV related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (i.e., ICAM-1, rel A, TNF α , p210^{bcr-abl} or RSV) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will

decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

II. Chemical Synthesis Of Ribozymes

- 5 There follows the chemical synthesis, deprotection, and purification of RNA, enzymatic RNA or modified RNA molecules in greater than milligram quantities with high biological activity. Applicant has determined that the synthesis of enzymatically active RNA in high yield and quantity is dependent upon certain critical steps used during its preparation.
- 10 Specifically, it is important that the RNA phosphoramidites are coupled efficiently in terms of both yield and time, that correct exocyclic amino protecting groups be used, that the appropriate conditions for the removal of the exocyclic amino protecting groups and the alkylsilyl protecting groups on the 2'-hydroxyl are used, and that the correct work-up and
- 15 purification procedure of the resulting ribozyme be used.

- To obtain a correct synthesis in terms of yield and biological activity of a large RNA molecule (*i.e.*, about 30 to 40 nucleotide bases), the protection of the amino functions of the bases requires either amide or substituted amide protecting groups, which must be, on the one hand, stable enough
- 20 to survive the conditions of synthesis, and on the other hand, removable at the end of the synthesis. These requirements are met by the amide protecting groups shown in Figure 8, in particular, benzoyl for adenosine, isobutyryl or benzoyl for cytidine, and isobutyryl for guanosine, which may be removed at the end of the synthesis by incubating the RNA in NH_3/EtOH
- 25 (ethanolic ammonia) for 20 h at 65 °C. In the case of the phenoxyacetyl type protecting groups shown in Figure 8 on guanosine and adenosine and acetyl protecting groups on cytidine, an incubation in ethanolic ammonia for 4 h at 65 °C is used to obtain complete removal of these protecting groups. Removal of the alkylsilyl 2'-hydroxyl protecting groups
- 30 can be accomplished using a tetrahydrofuran solution of TBAF at room temperature for 8-24 h.

- The most quantitative procedure for recovering the fully deprotected RNA molecule is by either ethanol precipitation, or an anion exchange cartridge desalting, as described in Scaringe *et al. Nucleic Acids Res.*
- 35 1990, 18, 5433-5341. The purification of the long RNA sequences may be

accomplished by a two-step chromatographic procedure in which the molecule is first purified on a reverse phase column with either the trityl group at the 5' position on or off. This purification is accomplished using an acetonitrile gradient with triethylammonium or bicarbonate salts as the aqueous phase. In the case of the trityl on purification, the trityl group may be removed by the addition of an acid and drying of the partially purified RNA molecule. The final purification is carried out on an anion exchange column, using alkali metal perchlorate salt gradients to elute the fully purified RNA molecule as the appropriate metal salts, e.g. Na⁺, Li⁺ etc. A final de-salting step on a small reverse-phase cartridge completes the purification procedure. Applicant has found that such a procedure not only fails to adversely affect activity of a ribozyme, but may improve its activity to cleave target RNA molecules.

Applicant has also determined that significant (see Tables 39-41) improvements in the yield of desired full length product (FLP) can be obtained by:

1. Using 5-S-alkyltetrazole at a delivered or effective concentration of 0.25-0.5 M or 0.15-0.35 M for the activation of the RNA (or analogue) amidite during the coupling step. (By delivered is meant that the actual amount of chemical in the reaction mix is known. This is possible for large scale synthesis since the reaction vessel is of size sufficient to allow such manipulations. The term effective means that available amount of chemical actually provided to the reaction mixture that is able to react with the other reagents present in the mixture. Those skilled in the art will recognize the meaning of these terms from the examples provided herein.) The time for this step is shortened from 10-15 m, *vide supra*, to 5-10 m. Alkyl, as used herein, refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to

7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which have an
5 unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the
10 substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated π electron
15 system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as
20 described above. Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur,
25 and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

30 2. Using 5-S-alkyltetrazole at an effective, or final, concentration of 0.1-0.35 M for the activation of the RNA (or analogue) amidite during the coupling step. The time for this step is shortened from 10-15 m, *vide supra*, to 5-10 m.

35 3. Using alkylamine (MA, where alkyl is preferably methyl, ethyl, propyl or butyl) or NH₄OH/alkylamine (AMA, with the same preferred alkyl groups as noted for MA) @ 65 °C for 10-15 m to remove the exocyclic

amino protecting groups (vs 4-20 h @ 55-65 °C using $\text{NH}_4\text{OH}/\text{EtOH}$ or NH_3/EtOH , *vide supra*). Other alkylamines, e.g. ethylamine, propylamine, butylamine *etc.* may also be used.

4. Using anhydrous triethylamine•hydrogen fluoride ($\text{aHF}\cdot\text{TEA}$)
5 @ 65 °C for 0.5-1.5 h to remove the 2'-hydroxyl alkylsilyl protecting group
(vs 8 - 24 h using TBAF, *vide supra* or $\text{TEA}\cdot 3\text{HF}$ for 24 h (Gasparutto *et al.*
Nucleic Acids Res. 1992, 20, 5159-5166). Other alkylamine•HF
complexes may also be used, e.g. trimethylamine or diisopropylethylamine.

5. The use of anion-exchange resins to purify and/or analyze the
10 fully deprotected RNA. These resins include, but are not limited to,
quaternary or tertiary amino derivatized stationary phases such as silica or
polystyrene. Specific examples include Dionex-NA100®, Mono-Q®, Poros-
Q®.

- Thus, the invention features an improved method for the coupling of
15 RNA phosphoramidites; for the removal of amide or substituted amide
protecting groups; and for the removal of 2'-hydroxyl alkylsilyl protecting
groups. Such methods enhance the production of RNA or analogs of the
type described above (e.g., with substituted 2'-groups), and allow efficient
synthesis of large amounts of such RNA. Such RNA may also have
20 enzymatic activity and be purified without loss of that activity. While specific
examples are given herein, those in the art will recognize that equivalent
chemical reactions can be performed with the alternative chemicals noted
above, which can be optimized and selected by routine experimentation.

- In another aspect, the invention features an improved method for the
25 purification or analysis of RNA or enzymatic RNA molecules (e.g. 28-70
nucleotides in length) by passing said RNA or enzymatic RNA molecule
over an HPLC, e.g., reverse phase and/or an anion exchange
chromatography column. The method of purification improves the catalytic
activity of enzymatic RNAs over the gel purification method (see Figure 10).

- 30 Draper *et al.*, PCT WO93/23569, incorporated by reference herein,
disclosed reverse phase HPLC purification. The purification of long RNA
molecules may be accomplished using anion exchange chromatography,
particularly in conjunction with alkali perchlorate salts. This system may be
used to purify very long RNA molecules. In particular, it is advantageous to

use a Dionex NucleoPak 100[®] or a Pharmacia Mono Q[®] anion exchange column for the purification of RNA by the anion exchange method. This anion exchange purification may be used following a reverse-phase purification or prior to reverse phase purification. This method results in the formation of a sodium salt of the ribozyme during the chromatography. Replacement of the sodium alkali earth salt by other metal salts, *e.g.*, lithium, magnesium or calcium perchlorate, yields the corresponding salt of the RNA molecule during the purification.

In the case of the 2-step purification procedure, in which the first step is a reverse phase purification followed by an anion exchange step, the reverse phase purification is best accomplished using polymeric, *e.g.* polystyrene based, reverse-phase media, using either a 5'-trityl-on or 5'-trityl-off method. Either molecule may be recovered using this reverse-phase method, and then, once detritylated, the two fractions may be pooled and then submitted to an anion exchange purification step as described above.

The method includes passing the enzymatically active RNA molecule over a reverse phase HPLC column; the enzymatically active RNA molecule is produced in a synthetic chemical method and not by an enzymatic process; and the enzymatic RNA molecule is partially blocked, and the partially blocked enzymatically active RNA molecule is passed over a reverse phase HPLC column to separate it from other RNA molecules.

In more preferred embodiments, the enzymatically active RNA molecule, after passage over the reverse phase HPLC column, is deprotected and passed over a second reverse phase HPLC column (which may be the same as the reverse phase HPLC column), to remove the enzymatic RNA molecule from other components. In addition, the column is a silica or organic polymer-based C4, C8 or C18 column having a porosity of at least 125 Å, preferably 300 Å, and a particle size of at least 2 µm, preferably 5 µm.

Activation

The synthesis of RNA molecules may be accomplished chemically or enzymatically. In the case of chemical synthesis the use of tetrazole as an activator of RNA phosphoramidites is known (Usman *et al.* *J. Am. Chem.*

Soc. 1987, 109, 7845-7854). In this, and subsequent reports, a 0.5 M solution of tetrazole is allowed to react with the RNA phosphoramidite and couple with the polymer bound 5'-hydroxyl group for 10 m. Applicant has determined that using 0.25-0.5 M solutions of 5-S-alkyltetrazoles for only 5 min gives equivalent or better results. The following exemplifies the procedure.

Example 7: Synthesis of RNA and Ribozymes Using 5-S-Alkyltetrazoles as Activating Agent

The method of synthesis used follows the general procedure for RNA synthesis as described in Usman et al., 1987 *supra* and in Scaringe et al., *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The major difference used was the activating agent, 5-S-ethyl or -methyltetrazole @ 0.25 M concentration for 5 min.

All small scale syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 μ mol scale protocol with a reduced 5 min coupling step for alkylsilyl protected RNA and 2.5 m coupling step for 2'-O-methylated RNA. A 6.5-fold excess (162.5 μ L of 0.1 M = 32.5 μ mol) of phosphoramidite and a 40-fold excess of S-ethyl tetrazole (400 μ L of 0.25 M = 100 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% N-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from Applied Biosystems.

All large scale syntheses were conducted on a modified (eight amidite port capacity) 390Z (ABI) synthesizer using a 25 μ mol scale protocol with a 5-15 min coupling step for alkylsilyl protected RNA and 7.5 m coupling step for 2'-O-methylated RNA. A six-fold excess (1.5 mL of 0.1 M = 150 μ mol) of phosphoramidite and a forty-five-fold excess of S-ethyl tetrazole (4.5 mL of

0.25 M = 1125 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 390Z, determined by colorimetric quantitation of the trityl fractions, was 95.0-96.7%. Oligonucleotide synthesis reagents for the 390Z: Detritylation solution was 2% DCA in methylene chloride; capping was performed with 16% *N*-Methylimidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. *S*-Ethyl tetrazole solution (0.25-0.5 M in acetonitrile) was made up from the solid obtained from Applied Biosystems.

Deprotection

The first step of the deprotection of RNA molecules may be accomplished by removal of the exocyclic amino protecting groups with either NH₄OH/EtOH:3/1 (Usman *et al.* *J. Am. Chem. Soc.* 1987, 109, 7845-7854) or NH₃/EtOH (Scarange *et al.* *Nucleic Acids Res.* 1990, 18, 5433-5341) for ~20 h @ 55-65 °C. Applicant has determined that the use of methylamine or NH₄OH/methylamine for 10-15 min @ 55-65 °C gives equivalent or better results. The following exemplifies the procedure.

Example 8: RNA and Ribozyme Deprotection of Exocyclic Amino Protecting Groups Using Methylamine (MA) or NH₄OH/Methylamine (AMA)

The polymer-bound oligonucleotide, either trityl-on or off, was suspended in a solution of methylamine (MA) or NH₄OH/methylamine (AMA) @ 55-65 °C for 5-15 min to remove the exocyclic amino protecting groups. The polymer-bound oligoribonucleotide was transferred from the synthesis column to a 4 mL glass screw top vial. NH₄OH and aqueous methylamine were pre-mixed in equal volumes. 4 mL of the resulting reagent was added to the vial, equilibrated for 5 m at RT and then heated at 55 or 65 °C for 5-15 min. After cooling to -20 °C, the supernatant was removed from the polymer support. The support was washed with 1.0 mL of EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder. The same procedure was followed for the aqueous methylamine reagent.

Table 40 is a summary of the results obtained using the improvements outlined in this application for base deprotection.

The second step of the deprotection of RNA molecules may be accomplished by removal of the 2'-hydroxyl alkylsilyl protecting group using TBAF for 8-24 h (Usman *et al. J. Am. Chem. Soc.* 1987, 109, 7845-7854). Applicant has determined that the use of anhydrous TEA•HF in *N*-methylpyrrolidine (NMP) for 0.5-1.5 h @ 55-65 °C gives equivalent or better results. The following exemplifies this procedure.

Example 9: RNA and Ribozyme Deprotection of 2'-Hydroxyl Alkylsilyl Protecting Groups Using Anhydrous TEA•HF

To remove the alkylsilyl protecting groups, the ammonia-deprotected oligoribonucleotide was resuspended in 250 µL of 1.4 M anhydrous HF solution (1.5 mL *N*-methylpyrrolidine, 750 µL TEA and 1.0 mL TEA•3HF) and heated to 65 °C for 1.5 h. 9 mL of 50 mM TEAB was added to quench the reaction. The resulting solution was loaded onto a Qiagen 500® anion exchange cartridge (Qiagen Inc.) prewashed with 10 mL of 50 mM TEAB. After washing the cartridge with 10 mL of 50 mM TEAB, the RNA was eluted with 10 mL of 2 M TEAB and dried down to a white powder.

Table 41 is a summary of the results obtained using the improvements outlined in this application for alkylsilyl deprotection.

Example 10: HPLC Purification, Anion Exchange column

For a small scale synthesis, the crude material was diluted to 5 mL with diethylpyrocarbonate treated water. The sample was injected onto either a Pharmacia Mono Q® 16/10 or Dionex NucleoPac® column with 100% buffer A (10 mM NaClO₄). A gradient from 180-210 mM NaClO₄ at a rate of 0.85 mM/void volume for a Pharmacia Mono Q® anion-exchange column or 100-150 mM NaClO₄ at a rate of 1.7 mM/void volume for a Dionex NucleoPac® anion-exchange column was used to elute the RNA. Fractions were analyzed by a HP-1090 HPLC with a Dionex NucleoPac® column. Fractions containing full length product at ≥80% by peak area were pooled.

For a trityl-off large scale synthesis, the crude material was desalted by applying the solution that resulted from quenching of the desilylation reaction to a 53 mL Pharmacia HiLoad 26/10 Q-Sepharose® Fast Flow column. The column was thoroughly washed with 10 mM sodium perchlorate buffer. The oligonucleotide was eluted from the column with

300 mM sodium perchlorate. The eluent was quantitated and an analytical HPLC was run to determine the percent full length material in the synthesis. The eluent was diluted four fold in sterile H₂O to lower the salt concentration and applied to a Pharmacia Mono Q[®] 16/10 column. A
5 gradient from 10-185 mM sodium perchlorate was run over 4 column volumes to elute shorter sequences, the full length product was then eluted in a gradient from 185-214 mM sodium perchlorate in 30 column volumes. The fractions of interest were analyzed on a HP-1090 HPLC with a Dionex NucleoPac[®] column. Fractions containing over 85% full length material
10 were pooled. The pool was applied to a Pharmacia RPC[®] column for desalting.

For a trityl-on large scale synthesis, the crude material was desalted by applying the solution that resulted from quenching of the desilylation reaction to a 53 mL Pharmacia HiLoad 26/10 Q-Sepharose[®] Fast Flow
15 column. The column was thoroughly washed with 20 mM NH₄CO₃H/10% CH₃CN buffer. The oligonucleotide was eluted from the column with 1.5 M NH₄CO₃H/10% acetonitrile. The eluent was quantitated and an analytical HPLC was run to determine the percent full length material present in the synthesis. The oligonucleotide was then applied to a Pharmacia Resource
20 RPC column. A gradient from 20-55% B (20 mM NH₄CO₃H/25% CH₃CN, buffer A = 20 mM NH₄CO₃H/10% CH₃CN) was run over 35 column volumes. The fractions of interest were analyzed on a HP-1090 HPLC with a Dionex NucleoPac[®] column. Fractions containing over 60% full length material were pooled. The pooled fractions were then submitted to manual
25 detritylation with 80% acetic acid, dried down immediately, resuspended in sterile H₂O, dried down and resuspended in H₂O again. This material was analyzed on a HP 1090-HPLC with a Dionex NucleoPac[®] column. The material was purified by anion exchange chromatography as in the trityl-off scheme (*vide supra*).

30 Example 11 Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 μM, 200 nM, 40 nM or 8 nM and the final substrate RNA
35 concentrations were ~ 1 nM. Total reaction volumes were 50 μL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were

initiated by mixing substrate and ribozyme solutions at $t = 0$. Aliquots of 5 μL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each aliquot was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis. Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

Example 12: One pot deprotection of RNA

Applicant has shown that aqueous methyl amine is an efficient reagent to deprotect bases in an RNA molecule. However, in a time consuming step (2-24 hrs), the RNA sample needs to be dried completely prior to the deprotection of the sugar 2'-hydroxyl groups. Additionally, deprotection of RNA synthesized on a large scale (e.g., 100 μmol) becomes challenging since the volume of solid support used is quite large. In an attempt to minimize the time required for deprotection and to simplify the process of deprotection of RNA synthesized on a large scale, applicant describes a one pot deprotection protocol (Fig. 12). According to this protocol, anhydrous methylamine is used in place of aqueous methyl amine. Base deprotection is carried out at 65 °C for 15 min and the reaction is allowed to cool for 10 min. Deprotection of 2'-hydroxyl groups is then carried out in the same container for 90 min in a TEA•3HF reagent. The reaction is quenched with 16 mM TEAB solution.

Referring to Fig. 13, hammerhead ribozyme targeted to site B is synthesized using RNA phosphoramidite chemistry and deprotected using either a two pot or a one pot protocol. Profiles of these ribozymes on an HPLC column are compared. The figure shows that RNAs deprotected by either the one pot or the two pot protocols yield similar full-length product profiles. Applicant has shown that using a one pot deprotection protocol, time required for RNA deprotection can be reduced considerably without compromising the quality or the yield of full length RNA.

Referring to Fig. 14, hammerhead ribozymes targeted to site B (from Fig. 13) are tested for their ability to cleave RNA. As shown in the figure 14, ribozymes that are deprotected using one pot protocol have catalytic activity comparable to ribozymes that are deprotected using a two pot protocol.

Example 12a: Improved protocol for the synthesis of phosphorothioate containing RNA and ribozymes using 5-S-Alkyltetrazoles as Activating Agent

5 The two sulfurizing reagents that have been used to synthesize ribophosphorothioates are tetraethylthiuram disulfide (TETD; Vu and Hirschbein, 1991 *Tetrahedron Letter* 31, 3005), and 3H-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage reagent; Vu and Hirschbein, 1991 *supra*). TETD requires long sulfurization times (600 seconds for DNA and 3600 seconds for RNA). It has recently been shown that for sulfurization of DNA
10 oligonucleotides, Beaucage reagent is more efficient than TETD (Wyrzykiewicz and Ravikumar, 1994 *Bioorganic Med. Chem.* 4, 1519). Beaucage reagent has also been used to synthesize phosphorothioate oligonucleotides containing 2'-deoxy-2'-fluoro modifications wherein the wait time is 10 min (Kawasaki et al., 1992 *J. Med. Chem.*).

15 The method of synthesis used follows the procedure for RNA synthesis as described herein and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The sulfurization step for RNA described in the literature is a 8 second delivery and 10 min wait steps (Beaucage
20 and Iyer, 1991 *Tetrahedron* 49, 6123). These conditions produced about 95% sulfurization as measured by HPLC analysis (Morvan et al., 1990 *Tetrahedron Letter* 31, 7149). This 5% contaminating oxidation could arise from the presence of oxygen dissolved in solvents and/or slow release of traces of iodine adsorbed on the inner surface of delivery lines during
25 previous synthesis.

A major improvement is the use of an activating agent, 5-S-ethyltetrazole or 5-S-methyltetrazole at a concentration of 0.25 M for 5 min. Additionally, for those linkages which are phosphorothioate, the iodine solution is replaced with a 0.05 M solution of 3H-1,2-benzodithiole-3-one
30 1,1-dioxide (Beaucage reagent) in acetonitrile. The delivery time for the sulfurization step is reduced to 5 seconds and the wait time is reduced to 300 seconds.

RNA synthesis is conducted on a 394 (ABI) synthesizer using a modified 2.5 μ mol scale protocol with a reduced 5 min coupling step for
35 alkylsilyl protected RNA and 2.5 min coupling step for 2'-O-methylated RNA. A 6.5-fold excess (162.5 μ L of 0.1 M = 32.5 μ mol) of phosphoramidite

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and a 40-fold excess of *S*-ethyl tetrazole (400 μ L of 0.25 M = 100 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394 synthesizer, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394 synthesizer: detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% *N*-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I_2 , 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. *S*-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from Applied Biosystems. Sulfurizing reagent was obtained from Glen Research.

Average sulfurization efficiency (ASE) is determined using the formula: $ASE = (PS/Total)^{1/n-1}$

where, PS = integrated ^{31}P NMR values of the P=S diester

Total = integration value of all peaks

n = length of oligo

Referring to tables 42 and 43, effects of varying the delivery and the wait time for sulfurization with Beaucage's reagent is described. These data suggest that 5 second wait time and 300 second delivery time is the condition under which ASE is maximum.

Using the above conditions a 36 mer hammerhead ribozyme is synthesized which is targeted to site C. The ribozyme is synthesized to contain phosphorothioate linkages at four positions towards the 5' end. RNA cleavage activity of this ribozyme is shown in Fig. 16. Activity of the phosphorothioate ribozyme is comparable to the activity of a ribozyme lacking any phosphorothioate linkages.

Example 13: Protocol for the synthesis of 2'-N-phthalimido-nucleoside phosphoramidite

The 2'-amino group of a 2'-deoxy-2'-amino nucleoside is normally protected with *N*-(9-fluorenylmethoxycarbonyl) (Fmoc; Imazawa and Eckstein, 1979 *supra*; Pleken et al., 1991 *Science* 253, 314). This protecting group is not stable in CH_3CN solution or even in dry form during

prolonged storage at -20 °C. These problems need to be overcome in order to achieve large scale synthesis of RNA.

Applicant describes the use of alternative protecting groups for the 2'-amino group of 2'-deoxy-2'-amino nucleoside. Referring to Figure 17,
5 phosphoramidite 17 was synthesized starting from 2'-deoxy-2'-aminonucleoside (12) using transient protection with Markievich reagent (Markiewicz *J. Chem. Res.* 1979, S, 24). An intermediate 13 was obtained in 50% yield, however subsequent introduction of N-phthaloyl (Pht) group by
10 Neffken's method (Neffkens, 1960 *Nature* 185, 306), desilylation (15), dimethoxytrytilation (16) and phosphitylation led to phosphoramidite 17. Since overall yield of this multi-step procedure was low (20%) applicant investigated some alternative approaches, concentrating on selective introduction of N-phthaloyl group without acylation of 5' and 3' hydroxyls.

When 2'-deoxy-2'-amino-nucleoside was reacted with 1.05
15 equivalents of Neffkens reagent in DMF overnight with subsequent treatment with Et₃N (1 hour) only 10-15% of N and 5'(3')-bis-phthaloyl derivatives were formed with the major component being N-Pht-derivative 15. The N,O-bis by-products could be selectively and quantitatively converted to N-Pht derivative 15 by treatment of crude reaction mixture
20 with cat. KCN/MeOH.

A convenient "one-pot" procedure for the synthesis of key intermediate 16 involves selective N-phthaloylation with subsequent dimethoxytrytilation by DMTCl/Et₃N and resulting in the preparation of DMT derivative 16 in 85% overall yield as follows. Standard phosphitylation of
25 16 produced phosphoramidite 17 in 87% yield. One gram of 2'-amino nucleoside, for example 2'-amino uridine (US Biochemicals® part # 77140) was co-evaporated twice from dry dimethyl formamide (Dmf) and dried in vacuo overnight. 50 mls of Aldrich sure-seal Dmf was added to the dry 2'-amino uridine via syringe and the mixture was stirred for 10 minutes
30 to produce a clear solution. 1.0 grams (1.05 eq.) of N-carbethoxyphthalimide (Neffken's reagent, 98% Janssen Chimica) was added and the solution was stirred overnight. Thin layer chromatography (TLC) showed 90% conversion to a faster moving products (10% ETOH in C HCl₃) and 57 µl of TEA (0.1 eq.) was added to effect closure of the
35 phthalimide ring. After 1 hour an additional 855 µl (1.5 eq.) of TEA was added followed by the addition of 1.53 grams (1.1 eq.) of DMT-Cl

(Lancaster Synthesis®, 98%). The reaction mixture was left to stir overnight and quenched with ETOH after TLC showed greater than 90% desired product. Dmf was removed under vacuum and the mixture was washed with sodium bicarbonate solution (5% aq., 500 mls) and extracted with ethyl acetate (2x 200 mls). A 25mm x 300mm flash column (75 grams Merck flash silica) was used for purification. Compound eluted at 80 to 85% ethyl acetate in hexanes (yield: 80% purity: >95% by ¹HNMR). Phosphoramidites were then prepared using standard protocols described above.

10 With phosphoramidite 17 in hand applicant synthesized several ribozymes with 2'-deoxy-2'-amino modifications. Analysis of the synthesis demonstrated coupling efficiency in 97-98% range. RNA cleavage activity of ribozymes containing 2'-deoxy-2'-amino-U modifications at U4 and/or U7 positions (see Figure 1), wherein the 2'-amino positions were either
15 protected with Fmoc or Pht, was identical. Additionally, complete deprotection of 2'-deoxy-2'-amino-Uridine was confirmed by base-composition analysis. The coupling efficiency of phosphoramidite 17 was not effected over prolonged storage (1-2 months) at low temperatures.

Protecting 2' Position with a SEM Group

20 There follows a method using the 2'-(trimethylsilyl)ethoxymethyl protecting group (SEM) in the synthesis of oligoribonucleotides, and in particular those enzymatic molecules described above. For the synthesis of RNA it is important that the 2'-hydroxyl protecting group be stable throughout the various steps of the synthesis and base deprotection. At the
25 same time, this group should also be readily removed when desired. To that end the *t*-butyldimethylsilyl group has been efficacious (Usman,N.; Oglivie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* 1990, 18, 5433-5441). However, long exposure times to tetra-*n*-butylammonium fluoride (TBAF) are generally required to fully remove this
30 protecting group from the 2'-hydroxyl. In addition, the bulky alkyl substituents can prove to be a hindrance to coupling thereby necessitating longer coupling times. Finally, it has been shown that the TBDMS group is base labile and is partially deprotected during treatment with ethanolic ammonia (Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* 1990,
35

18, 5433-5441 and Stawinski, J.; Stromberg, R.; Thelin, M.; Westman, E. *Nucleic Acids Res.* 1988, 16, 9285-9298).

5 The (trimethylsilyl)ethoxymethyl ether (SEM) seems a suitable substitute. This protecting group is stable to base and all but the harshest acidic conditions. Therefore it is stable under the conditions required for oligonucleotide synthesis. It can be readily introduced and the oxygen carbon bond makes it unable to migrate. Finally, the SEM group can be removed with $\text{BF}_3 \cdot \text{OEt}_2$ very quickly.

10 There follows a method for synthesis of RNA by protecting the 2'-position of a nucleotide during RNA synthesis with a (trimethylsilyl)ethoxymethyl (SEM) group. The method can involve use of standard RNA synthesis conditions as discussed below, or any other equivalent steps. Those in the art are familiar with such steps. The nucleotide used can be any normal nucleotide or may be substituted in
15 various positions by methods well known in the art, e.g., as described by Eckstein *et al.*, *International Publication No. WO 92/07065*, Perrault *et al.*, *Nature* 1990, 344, 565-568, Pieken *et al.*, *Science* 1991, 253, 314-317, Usman, N.; Cedergren, R.J. *Trends in Biochem. Sci.* 1992, 17, 334-339, Usman *et al.*, PCT WO93/15187, and Sproat, B. *European Patent*
20 *Application 92110298.4*.

This invention also features a method for covalently linking a SEM group to the 2'-position of a nucleotide. The method involves contacting a nucleoside with an SEM-containing molecule under SEM bonding conditions. In a preferred embodiment, the conditions are dibutyltin oxide,
25 tetrabutylammonium fluoride and SEM-Cl. Those in the art, however, will recognize that other equivalent conditions can also be used.

In another aspect, the invention features a method for removal of an SEM group from a nucleoside molecule or an oligonucleotide. The method involves contacting the molecule or oligonucleotide with boron trifluoride
30 etherate ($\text{BF}_3 \cdot \text{OEt}_2$) under SEM removing conditions, e.g., in acetonitrile.

Referring to Figure 18, there is shown the method for solid phase synthesis of RNA. A 2',5'-protected nucleotide is contacted with a solid phase bound nucleotide under RNA synthesis conditions to form a dinucleotide. The protecting group (R) at the 2'-position in prior art

methods can be a silyl ether, as shown in the Figure. In the method of the present invention, an SEM group is used in place of the silyl ether. Otherwise RNA synthesis can be performed by standard methodology.

Referring to Figure 19, there is shown the synthesis of 2'-O-SEM protected nucleosides and phosphoramidites. Briefly, a 5'-protected nucleoside (1) is protected at the 2'- or 3'-position by contacting with a derivative of SEM under appropriate conditions. Specifically, those conditions include contacting the nucleoside with dibutyltin oxide and SEM chloride. The 2 regioisomers are separated by chromatography and the 2'-protected moiety is converted into a phosphoramidite by standard procedure. The 3'-protected nucleoside is converted into a succinate derivative suitable for derivatization of a solid support.

Referring to Figure 20, a prior art method for deprotection of RNA using silyl ethers is shown. This contrasts with the method shown in Figure 21 in which deprotection of RNA containing an SEM group is performed. In step 1, the base protecting groups and cyanoethyl groups are removed by standard procedure. The SEM group is then removed as shown in the Figure. The details of the synthesis of phosphoramidites and SEM protected nucleosides and their use in synthesis of oligonucleotides and subsequent deprotection of

Example 14: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5'-O-Di-methoxytrityl Uridine (2)

Referring to Figure 19, 5'-O-dimethoxytrityl uridine 1 (1.0 g, 1.83 mmol) in CH₃CN (18 mL) was added dibutyltin oxide (1.0 g, 4.03 mmol) and TBAF (1 M, 2.38 mL, 2.38 mmol). The mixture was stirred for 2 h at RT (about 20-25°C) at which time (trimethylsilyl)ethoxymethyl chloride (SEM-Cl) (487 µL, 2.75 mmol) was added. The reaction mixture was stirred overnight and then filtered and evaporated. Flash chromatography (30% hexanes in ethyl acetate) yielded 347 mg (28.0%) of 2'-hydroxyl protected nucleoside 2 and 314 mg (25.3%) of 3'-hydroxyl protected nucleoside 3.

Example 15: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl) Uridine (4)

Nucleoside 2 was detritylated following standard methods, as shown in Figure 19.

Example 16: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5',3'-O-Acetyl Uridine (5)

Nucleoside 4 was acetylated following standard methods, as shown in Figure 19.

5 Example 17: Synthesis of 5',3'-O-Acetyl Uridine (6)

Referring to Figure 19, the fully protected uridine 5 (32 mg, 0.07 mmol) was dissolved in CH₃CN (700 µL) and BF₃•OEt₂ (17.5 µL, 0.14 mmol) was added. The reaction was stirred 15 m and MeOH was added to quench the reaction. Flash chromatography (5% MeOH in CH₂Cl₂) gave
10 20 mg (88%) of SEM deprotected nucleoside 6.

Example 18: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-3'-O-Succinyl-5'-O-Dimethoxytrityl Uridine (2)

Nucleoside 3 was succinylated and coupled to the support following standard procedures, as shown in Figure 19.

15 Example 19: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5'-O-Dimethoxytrityl Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (8)

Nucleoside 3 was phosphitylated following standard methods, as shown in Figure 19.

20 Example 20: Synthesis of RNA Using 2'-O-SEM Protection

Referring to Figure 18, the method of synthesis used follows the general procedure for RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.*
25 1990, 18, 5433-5441. The phosphoramidite 8 was coupled following standard RNA methods to provide a 10-mer of uridylic acid. Syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 µmol scale protocol with a 10 m coupling step. A thirteen-fold excess (325 µL of 0.1 M = 32.5 µmol) of phosphoramidite and a 80-fold excess of tetrazole
30 (400 µL of 0.5 M = 200 µmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, were 98-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% N-

Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle.

- 5 Referring to Figure 21, the homopolymer was base deprotected with NH₃/EtOH at 65 °C. The solution was decanted and the support was washed twice with a solution of 1:1:1 H₂O:CH₃CN:MeOH. The combined solutions were dried down and then diluted with CH₃CN (1 mL). BF₃•OEt₂ (2.5 µL, 30 µmol) was added to the solution and aliquots were removed at
10 ten time points. The results indicate that after 30 min deprotection is complete, as shown in Figure 22.

III. Vectors Expressing Ribozymes

- There follows a method for expression of a ribozyme in a bacterial or eucaryotic cell, and for production of large amounts of such a ribozyme. In
15 general, the invention features a method for preparing multi-copy cassettes encoding a defined ribozyme structure for production of a ribozyme at a decreased cost. A vector is produced which encodes a plurality of ribozymes which are cleaved at their 3' and 5' ends from an RNA transcript produced from the vector by only one other ribozyme. The system is useful
20 for scaling up production of a ribozyme, which may be either modified or unmodified, *in situ* or *in vitro*. Such vector systems can be used to express a desired ribozyme in a specific cell, or can be used in an *in vitro* system to allow production of large amounts of a desired ribozyme. The vectors of this invention allow a higher yield synthesis of a ribozyme in the form of an
25 RNA transcript which is cleaved *in situ* or *in vitro* before or after transcript isolation.

- Thus, this invention is distinct from the prior art in that a single ribozyme is used to process the 3' and 5' ends of each therapeutic, trans-
acting or desired ribozyme instead of processing only one end, or only one
30 ribozyme. This allows smaller vectors to be derived with multiple trans-acting ribozymes released by only one other ribozyme from the mRNA transcript. Applicant has also provided methods by which the activity of such ribozymes is increased compared to those in the art, by designing ribozyme-encoding vectors and the corresponding transcript such that

folding of the mRNA does not interfere with processing by the releasing ribozyme.

The stability of the ribozyme produced in this method can be enhanced by provision of sequences at the termini of the ribozymes as described by Draper et al., PCT WO 93/23509, hereby incorporated by
5 reference herein.

The method of this invention is advantageous since it provides high yield synthesis of ribozymes by use of low cost transcription-based protocols, compared to existing chemical ribozyme synthesis, and can use
10 isolation techniques currently used to purify chemically synthesized oligonucleotides. Thus, the method allows synthesis of ribozymes in high yield at low cost for analytical, diagnostic, or therapeutic applications.

The method is also useful for synthesis of ribozymes *in vitro* for ribozyme structural studies, enzymatic studies, target RNA accessibility
15 studies, transcription inhibition studies and nuclease protection studies, much is described by Draper et al., PCT WO 93/23509 hereby incorporated by reference herein.

The method can also be used to produce ribozymes *in situ* either to increase the intracellular concentration of a desired therapeutic ribozyme,
20 or to produce a concatameric transcript for subsequent *in vitro* isolation of unit length ribozyme. The desired ribozyme can be used to inhibit gene expression in molecular genetic analyses or in infectious cell systems, and to test the efficacy of a therapeutic molecule or treat afflicted cells.

Thus, in general, the invention features a vector which includes a
25 bacterial, viral or eucaryotic promoter within a plasmid, cosmid, phagmid, virus, viroid, virusoid or phage vector. Other vectors are equally suitable and include double-stranded, or partially double-stranded DNA, formed by an amplification method such as the polymerase chain reaction, or double-stranded, partially double-stranded or single-stranded RNA, formed by site-
30 directed homologous recombination into viral or viroid RNA genomes. Such vectors need not be circular. Transcriptionally linked to the promoter region is a first ribozyme-encoding region, and nucleotide sequences encoding a ribozyme cleavage sequence which is placed on either side of a region encoding a therapeutic or otherwise desired second ribozyme.

Suitable restriction endonuclease sites can be provided to ease construction of this vector in DNA vectors or in requisite DNA vectors of an RNA expression system. The desired second ribozyme may be any desired type of ribozyme, such as a hammerhead, hairpin, hepatitis delta virus (HDV) or other catalytic center, and can include group I and group II introns, as discussed above. The first ribozyme is chosen to cleave the encoded cleavage sequence, and may also be any desired ribozyme, for example, a *Tetrahymena* derived ribozyme, which may, for example, include an imbedded restriction endonuclease site in the center of a self-recognition sequence to aid in vector construction. This endonuclease site is useful for construction of the vector, and subsequent analysis of the vector.

When the promoter of such a vector is activated an RNA transcript is produced which includes the first and second ribozyme sequences. The first ribozyme sequence is able to act, under appropriate conditions, to cause cleavage at the cleavage sites to release the second ribozyme sequences. These second ribozyme sequences can then act at their target RNA sites, or can be isolated for later use or analysis.

Thus, in one aspect the invention features a vector which includes a first nucleic acid sequence (encoding a first ribozyme having intramolecular cleaving activity), and a second nucleic acid sequence (encoding a second ribozyme having intermolecular cleaving enzymatic activity) flanked by nucleic acid sequences encoding RNA which is cleaved by the first ribozyme to release the second ribozyme from the RNA transcript encoded by the vector. The second ribozyme may be flanked by the first ribozyme either on the 5' side or 3' side. If desired, the first ribozyme may be encoded on a separate vector and may have intermolecular cleaving activity.

As discussed above, the first ribozyme can be chosen to be any self-cleaving ribozyme, and the second ribozyme may be chosen to be any desired ribozyme. The flanking sequences are chosen to include sequences recognized by the first ribozyme. When the vector is caused to express RNA from these nucleic acid sequences, that RNA has the ability under appropriate conditions to cleave each of the flanking regions and thereby release one or more copies of the second ribozyme. If desired, several different second ribozymes can be produced by the same vector, or

several different vectors can be placed in the same vessel or cell to produce different ribozymes.

In preferred embodiments, the vector includes a plurality of the nucleic acid sequences encoding the second ribozyme, each flanked by nucleic acid sequences recognized by the first ribozyme. Most preferably, such a plurality includes at least six to nine or even between 60 - 100 nucleic acid sequences. In other preferred embodiments, the vector includes a promoter which regulates expression of the nucleic acid encoding the ribozymes from the vector; and the vector is chosen from a plasmid, cosmid, phagemid, virus, viroid or phage. In a most preferred embodiment, the plurality of nucleic acid sequences are identical and are arranged in sequential order such that each has an identical end nearest to the promoter. If desired, a poly(A) sequence adjacent to the sequence encoding the first or second ribozyme may be provided to increase stability of the RNA produced by the vector; and a restriction endonuclease site adjacent to the nucleic acid encoding the first ribozyme is provided to allow insertion of nucleic acid encoding the second ribozyme during construction of the vector.

In a second aspect, the invention features a method for formation of a ribozyme expression vector by providing a vector including nucleic acid encoding a first ribozyme, as discussed above, and providing a single-stranded DNA encoding a second ribozyme, as discussed above. The single-stranded DNA is then allowed to anneal to form a partial duplex DNA which can be filled in by a treatment with an appropriate enzyme, such as a DNA polymerase in the presence of dNTPs, to form a duplex DNA which can then be ligated to the vector. Large vectors resulting from this method can then be selected to insure that a high copy number of the single-stranded DNA encoding the second ribozyme is incorporated into the vector.

In a further aspect, the invention features a method for production of ribozymes by providing a vector as described above, expressing RNA from that vector, and allowing cleavage by the first ribozyme to release the second ribozyme.

In preferred embodiments, three different ribozyme motifs are used as cis-cleaving ribozymes. The hammerhead, hairpin, and hepatitis delta

virus (HDV) ribozyme motifs consist of small, well-defined sequences that rapidly self-cleave *in vitro* (Symons, 1992 Annu. Rev. Biochem. 61, 641). While structural and functional differences exist among the three ribozyme motifs, they self-process efficiently *in vivo*. All three ribozyme motifs self-process to 87-95% completion in the absence of 3' flanking sequences. *In vitro*, the self-processing constructs described in this invention are significantly more active than those reported by Taira et al., 1990 supra; and Altschuler et al., 1992 Gene 122, 85. The present invention enables the use of cis-cleaving ribozymes to efficiently truncate RNA molecules at specific sites *in vivo* by ensuring lack of secondary structure which prevents processing.

Isolation of Therapeutic Ribozyme

The preferred method of isolating therapeutic ribozyme is by a chromatographic technique. The HPLC purification methods and reverse HPLC purification methods described by Draper et al., PCT WO 93/23509, hereby incorporated by reference herein, can be used. Alternatively, the attachment of complementary oligonucleotides to cellulose or other chromatography columns allows isolation of the therapeutic second ribozyme, for example, by hybridization to the region between the flanking arms and the enzymatic RNA. This hybridization will select against the short flanking sequences without the desired enzymatic RNA, and against the releasing first ribozyme. The hybridization can be accomplished in the presence of a chaotropic agent to prevent nuclease degradation. The oligonucleotides on the matrix can be modified to minimize nuclease activity, for example, by provision of 2'-O-methyl RNA oligonucleotides. Such modifications of the oligonucleotide attached to the column matrix will allow the multiple use of the column with minimal oligo degradation. Many such modifications are known in the art, but a chemically stable non-reducible modification is preferred. For example, phosphorothioate modifications can also be used.

The expressed ribozyme RNA can be isolated from bacterial or eucaryotic cells by routine procedures such as lysis followed by guanidine isothiocyanate isolation.

The current known self-cleaving site of *Tetrahymena* can be used in an alternative vector of this invention. If desired, the full-length

Tetrahymena sequence may be used, or a shorter sequence may be used. It is preferred that, in order to decrease the superfluous sequences in the self-cleaving site at the 5' cleavage end, the hairpin normally present in the *Tetrahymena* ribozyme should contain the therapeutic second ribozyme 3' sequence and its complement. That is, the first releasing ribozyme-encoding DNA is provided in two portions, separated by DNA encoding the desired second ribozyme. For example, if the therapeutic second ribozyme recognition sequence is CGGACGA/CGAGGA, then CGAGGA is provided in the self-cleaving site loop such that it is in a stem structure recognized by the *Tetrahymena* ribozyme. The loop of the stem may include a restriction endonuclease site into which the desired second ribozyme-encoding DNA is placed.

If desired, the vector may be used in a therapeutic protocol by use of the systems described by Lechner, PCT WO 92/13070, hereby incorporated by reference herein, to allow a timed expression of the therapeutic second ribozyme, as well as an appropriate shut off of cell or gene function. Thus, the vector will include a promoter which appropriately expresses enzymatically active RNA only in the presence of an RNA or another molecule which indicates the presence of an undesired organism or state. Such enzymatically active RNA will then kill or harm the cell in which it exists, as described by Lechner, *id.*, or act to cause reduced expression of a desired protein product.

A number of suitable RNA vectors may also be used in this invention. The vectors include plant viroids, plant viruses which contain single or double-stranded RNA genomes and animal viruses which contain RNA genomes, such as the picomaviruses, myxoviruses, paramyxoviruses, hepatitis A virus, reovirus and retroviruses. In many instances cited, use of these viral vectors also results in tissue specific delivery of the ribozymes.

Example 21: Design of self-processing cassettes

In a preferred embodiment, applicant compared the *in vitro* and *in vivo* cis-cleaving activity of three different ribozyme motifs—the hammerhead, the hairpin and the hepatitis delta virus ribozyme—in order to assess their potential to process the ends of transcripts *in vivo*. To make a direct comparison among the three, however, it is important to design the ribozyme-containing transcripts to be as similar as possible. To this end,

all the ribozyme cassettes contained the same trans-acting hammerhead ribozyme followed immediately by one of the three cis-acting ribozymes (Figure 23-25). For simplicity, applicant refers to each cassette by an abbreviation that indicates the downstream cis-cleaving ribozyme only.

- 5 Thus HH refers to the cis-cleaving cassette containing a hammerhead ribozyme, while HP and HDV refer to the cassettes containing hairpin and hepatitis delta virus cis-cleaving ribozymes, respectively. The general design of the ribozyme cassettes, as well as specific differences among the cassettes, are outlined below.

- 10 A sequence predicted to form a stable stem-loop structure is included at the 5' end of all the transcripts. The hairpin stem contains the T7 RNA polymerase initiation sequence (Milligan & Uhlenbeck, 1989 Methods Enzymol. 180, 51) and its complement, separated by a stable tetra-loop (Antao et al., 1991 Nucleic Acids Res. 19, 5901). By incorporating the T7
15 initiation sequence into a stem-loop structure, applicant hoped to avoid nonproductive base pairing interactions with either the trans-acting ribozyme or with the cis-acting ribozyme. The presence of a hairpin at the end of a transcript may also contribute to the stability of the transcript *in vivo*. These are non-limiting examples. Those in the art will recognize that
20 other embodiments can be readily generated using a variety of promoters, initiator sequences and stem-loop structure combinations generally known in the art.

- The trans-acting ribozyme used in this study is targeted to a site B (5'...CUGGAGUC↓GACCUUC...3'). The 5' binding arm of the ribozyme, 5'-
25 GAAGGUC-3', and the core of the ribozyme, 5'-CUGAUGAGGCCGAAAGGCCGAA-3', remain constant in all cases. In addition, all transcripts also contain a single nucleotide between the 5' stem-loop and the first nucleotide of the ribozyme. The linker nucleotide was required to obtain the same activity *in vitro* that was measured with an
30 identical ribozyme lacking the 5' hairpin. Because the three cis-cleaving ribozymes have different requirements at the site of cleavage, slight differences were unavoidable at the 3' end of the processed transcript. The junction between the trans- and cis-acting ribozyme is, however, designed so that there is minimal extraneous sequence left at the 3' end of the trans-
35 cleaving ribozyme once cis-cleavage occurs. The only differences between the constructs lie in the 3' binding arm of the ribozyme, where

either 6 or 7 nucleotides, 5'-ACUCCA(+/-G)-3', complementary to the target sequence are present and where, after processing, two to five extra nucleotides remain.

The cis-cleaving hammerhead ribozyme used in the HH cassette is based on the design of Grosshans and Cech, 1991 supra. As shown in Figure 23, the 3' binding arm of the trans-acting ribozyme is included in the required base-pairing interactions of the cis-cleaving ribozyme to form stem I. Two extra nucleotides, UC, were included at the end of the 3' binding arm to form the self-processing hammerhead ribozyme site (Ruffner et al., 1990 supra) which remain on the 3' end of the trans-acting ribozyme following self-processing.

The hairpin ribozyme portion of the HP self-processing construct is based on the minimal wild-type sequence (Hampel & Tritz, 1989 supra). A tetra-loop at the end of helix 1 (3' side of the cleavage site) serves to link the two portions and thus allows a minimal five nucleotides to remain at the end of the released trans-acting ribozyme following self-processing. Two variants of HP were designed: HP(GU) and HP(GC). The HP(GU) was constructed with a G-U wobble base pair in helix 2 (A52G substitution; Figure 24). This slight destabilization of helix 2 was intended to improve self-processing activity by promoting product release and preventing the reverse reaction (Berzal-Herranz et al., 1992 Genes & Dev. 6, 129; Chowrira et al., 1993 Biochemistry 32, 1088). The HP(GC) cassette was constructed as a control for strong base-pairing interactions in helix 2 (U77C and A52G substitution; Figure 24). Another modification to discourage the reverse ligation reaction of the hairpin ribozyme was to shorten helix 1 (Figure 24) by one base pair relative to the wild-type sequence (Chowrira & Burke, 1991 Biochemistry 30, 8518).

The HDV ribozyme self-processes efficiently when the nucleotide 5' to the cleavage site is a pyrimidine, and somewhat less so when adenosine is in that position. No other sequence requirements have been identified upstream of the cleavage site, however, we have observed some decrease in activity when a stem-loop structure was present within 2 nt of the cleavage site. The HDV self-processing construct (Fig 25) was designed to generate the trans-acting hammerhead ribozyme with only two additional nucleotides at its 3' end after self-processing. The HDV sequence used here is based on the anti-genomic sequence (Perrota & Been, 1992 supra)

but includes the modifications of Been et al., 1992 (Biochemistry 31, 11843) in which cis-cleavage activity of the ribozyme was improved by the substitution of a shortened helix 4 for a wild-type stem-loop (Figure 25).

5 To prepare DNA inserts that encode self-processing ribozyme cassettes, partially overlapping top- and bottom-strand oligonucleotides (60-90 nucleotides) were designed to include sequences for the T7 promoter, the trans-acting ribozyme, the cis-cleaving ribozyme and appropriate restriction sites for use in cloning (see Fig. 26). The single-strand portions of annealed oligonucleotides were converted to double-
10 strands using Sequenase® (U.S. Biochemicals). Insert DNA was ligated into *EcoRI/HindIII*-digested puc18 and transformed into *E. coli* strain DH5α using standard protocols (Maniatis et al., 1982 in Molecular Cloning Cold Spring Harbor Press). The identity of positive clones was confirmed by sequencing small-scale plasmid preparations.

15 Larger scale preparations of plasmid DNA for use as *in vitro* transcription templates and in transactions were prepared using the protocol and columns from QIAGEN Inc. (Studio City, CA) except that an additional ethanol precipitation was included as the final step.

Example 22: RNA Processing *in vitro*

20 Transcription reactions containing linear plasmid templates were carried out essentially as described (Milligan & Uhlenbeck, 1989 Supra; Chowrira & Burke, 1991 Supra). In order to prepare 5' end-labeled transcripts, standard transcription reactions were carried out in the presence of 10-20 μCi [γ -³²P]GTP, 200 μM each NTP and 0.5 to 1 μg of
25 linearized plasmid template. The concentration of MgCl₂ was maintained at 10 mM above the total nucleotide concentration.

To compare the ability of the different ribozyme cassettes to self-process *in vitro*, each construct was transcribed and allowed to undergo self-processing under identical conditions at 37°C. For these comparisons,
30 equal amounts of linearized DNA templates bearing the various ribozyme cassettes were transcribed in the presence of [γ -³²P]GTP to generate 5' end-labeled transcripts. In this manner only the full-length, unprocessed transcripts and the released trans-ribozymes are visualized by autoradiography. In all reactions, Mg²⁺ was included at 10 mM above the
35 nucleotide concentration so that cleavage by all the ribozyme cassettes

would be supported. Transcription templates were linearized at several positions by digestion with different restriction enzymes so that self-processing in the presence of increasing lengths of downstream sequence could be compared (see Fig. 26). The resulting transcripts have either 4-5
5 non-ribozyme nucleotides at the 3' end (*Hind*III-digested template), 220 nucleotides (*Nde*I digested templates) or 454 nucleotides of downstream sequence (*Rca*I digested template).

As shown in Figure 27, all four ribozyme cassettes are capable of self-processing and yield RNA products of expected sizes. Two nucleotides
10 essential for hammerhead ribozyme activity (Ruffner et al., 1990 *supra*) have been changed in the HH(mutant) core sequence (see Figure 23) and so this transcript is unable to undergo self-processing (Fig. 27). This is evidenced by the lack of a released 5' RNA in the HH(mutant), although the full-length RNAs are present. Comparison of the amounts of released
15 trans-ribozyme (Fig. 27) indicate that there are differences in the ability of these ribozymes to self-process *in vitro*, especially with respect to the presence of downstream sequence. For the two HP constructs, it is clear that HP(GC) is more efficient than the HP(GU) ribozyme, both in the presence and in the absence of extra downstream sequence. In addition,
20 the activity of HP(GU) falls off more dramatically when downstream sequence is present. The stronger G:C base pair likely contributes to the HP(GC) construct's ability to fold correctly (and/or more quickly) into the productive structure, even when as much as 216 extra nucleotides are present downstream. The HH ribozyme construct is also quite efficient at
25 self-processing, and slightly better than the HP(GU) construct even when downstream sequence is present.

Of the three ribozyme motifs, the presence of extra downstream sequence seems to most affect the efficiency of HDV. When no extra sequence is present downstream, HDV is quite efficient and self-processes
30 to approximately the same level as the HH and HP(GC) cassettes. However, when extra downstream sequence is present, the self-processing activity seems to decrease almost as dramatically as is seen with the (sub-optimal) HP(GU) cassette.

Example 23: Kinetics of self-processing reaction

HindIII-digested template (250 ng) was used in a standard transcription reaction mixture containing: 50 mM Tris-HCl pH 8.3; 1 mM ATP, GTP and UTP; 50 μ M CTP; 40 μ Ci [α - 32 P]CTP; 12 mM MgCl₂; 10 mM DTT. The transcription/self-processing reaction was initiated by the addition of T7 RNA polymerase (15 U/ μ l). Aliquots of 5 μ l were taken at regular time intervals and the reaction was stopped by adding an equal volume of 2x formamide loading buffer (95% formamide, 15 mM EDTA, & dyes) and freezing on dry ice. The samples were resolved on a 10% polyacrylamide sequencing gel and results were quantitated by PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Ribozyme self-cleavage rates were determined from non-linear, least-squares fits (KaleidaGraph, Synergy Software, Reading, PA) of the data to the equation:

$$(\text{Fraction Uncleaved Transcript}) = \frac{1}{kt} (1 - e^{-kt})$$

where t represents time and k represents the unimolecular rate constant for cleavage (Long & Uhlenbeck, 1994 Proc. Natl. Acad. Sci. USA 91, 6977).

Linear templates were prepared by digesting the plasmids with *HindIII* so that transcripts will contain only four to five vector-derived nucleotides at the 3' end (see Figure 23-25). By comparison of the unimolecular rate constant (k) determined for each construct, it is clear that HH is the most efficient at self-processing (Table 44). The HH transcript self-processes 2-fold faster than HDV and 3-fold faster than HP(GC) transcripts. Although the HP(GU) RNA undergoes self-processing, it is at least 6-fold slower than the HP(GC) construct. This is consistent with previous observations that the stability of helix 2 is essential for self-processing and trans-cleavage activity of the hairpin ribozyme (Hampel et al., 1990 supra; Chowrira & Burke, 1991 supra). The rate of HH self-cleavage during transcription measured here (1.2 min^{-1}) is similar to the rate measured by Long and Uhlenbeck 1994 supra using a HH that has a different stem I and stem III. Self-processing rates during transcription for HP and HDV have not been previously reported. However, self-processing of the HDV ribozyme—as measured here during transcription—is significantly slower than when tested after isolation from a denaturing gel (Been et al., 1992 supra). This decrease likely reflects the difference in protocol as well as the presence of 5' flanking sequence in the HDV construct used here.

Example 24: Effect of downstream sequences on trans-cleavage *in vitro*

Transcripts containing the trans ribozyme with or without 3' flanking sequences were assayed for their ability to cleave their target *in trans*. To this end, transcripts from three templates were resolved on a preparative gel and bands corresponding both to processed trans-acting ribozymes from the HH transcription reaction, and to full-length HH(mutant) and Δ HDV transcripts were isolated. In all three transcripts the trans-acting ribozyme portion is identical with the exception of sequences at their 3' ends. The HH trans-acting ribozyme contains only an additional UC at its 3' end, while HH(mutant) and Δ HDV have 52 and 37 nucleotides, respectively, at their 3' ends. A 622 nucleotide, internally-labeled target RNA was incubated, under ribozyme excess conditions, along with the three ribozyme transcripts in a standard reaction buffer.

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 622 nt region (containing hammerhead site P) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed in a standard transcription buffer in the presence of [α - 32 P]CTP (Chowrira & Burke, 1991 *supra*). The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed by chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (1 μ M) and internally labeled 622 nt substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager[®] (Molecular Dynamics, Sunnyvale, CA).

The HH trans-acting ribozyme cleaves the target RNA approximately 10-fold faster than the Δ HDV transcript and greater than 20-fold faster than

the HH(mutant) transcript (Figure 28). The additional nucleotides at the end of HH(mutant) form 7 base-pairs with the 3' target-binding arm of the trans-acting ribozyme (Figure 23). This interaction must be disrupted (at a cost of 6 kcal/mole) to make the trans-acting ribozyme available for binding the target sequence. In contrast, the additional nucleotides at the end of Δ HDV were not designed to form any strong, alternative base-pairing with the trans-ribozyme. Nevertheless, the Δ HDV sequences are predicted to form multiple structures involving the 3' target-binding arm of the trans ribozyme that have stabilities ranging from 1-2 kcal/mole. Thus, the observed reductions in activity for the Δ HDV and HH(mutant) constructs are consistent with the predicted folded structures, and it reinforces the view that the flanking sequences can decrease the catalytic efficiency of a ribozyme through nonproductive interactions with either the ribozyme or the substrate or both.

Example 25: RNA self-processing *in vivo*

Since three of the constructs (HH, HDV and HP(GC)) self-process efficiently in solution, the affect of the mammalian cellular milieu on ribozyme self-processing was next explored by applicant. A transient expression system was employed to investigate ribozyme activity *in vivo*. A mouse cell line (OST7-1) that constitutively expresses T7 RNA polymerase in the cytoplasm was chosen for this study (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. USA 87, 6743). In these cells plasmids containing a ribozyme cassette downstream of the T7 promoter will be transcribed efficiently in the cytoplasm (Elroy-Stein & Moss, 1990 supra).

Monolayers of a mouse L9 fibroblast cell line (OST7-1; Elroy-Stein and Moss, 1990 supra) were grown in 6-well plates with $\sim 5 \times 10^5$ cells/well. Cells were transfected with circular plasmids (5 μ g/well) using the calcium phosphate-DNA precipitation method (Maniatis et al., 1982 supra). Cells were lysed (4 hours post-transfection) by the addition of standard lysis buffer (200 μ l/well) containing 4M guanadinium isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarkosyl (Chomczynski and Sacchi, 1987 Anal. Biochem. 162, 156), and 50 mM EDTA pH 8.0. The lysate was extracted once with water-saturated phenol followed by one extraction with chloroform:isoamyl alcohol (25:1). Total cellular RNA was precipitated with an equal volume of isopropanol. The RNA pellet was resuspended in 0.2

M ammonium acetate and reprecipitated with ethanol. The pellet was then washed with 70% ethanol and resuspended in DEPC-treated water.

Purified cellular RNA (3 µg/reaction) was first denatured in the presence of a 5' end-labeled DNA primer (100 pmol) by heating to 90°C for 2 min. in the absence of Mg²⁺, and then snap-cooling on ice for at least 15 min. This protocol allows for efficient annealing of the primer to its complementary RNA sequence. The primer was extended using Superscript II reverse transcriptase (8 U/µl; BRL) in a buffer containing 50 mM Tris·HCl pH 8.3; 10 mM DTT; 75 mM KCl; 1 mM MgCl₂; 1 mM each dNTP. The extension reaction was carried out at 42°C for 10 min. The reaction was terminated by adding an equal volume of 2x formamide gel loading buffer and freezing on crushed dry ice. The samples were resolved on a 10% polyacrylamide sequencing gel. The primer sequences are as follows: HH primer, 5'-CTCCAGTTTCGAGCTTT-3'; HDV primer, 5'-AAGTAGCCCAGGTCCGACC-3'; HP primer, 5'-ACCAGGTAATATACCACAAC-3'.

As shown in Figure 29, specific bands corresponding to full-length precursor RNA and 3' cleavage products were detected from cells transfected with the self-processing cassettes. All three constructs, in addition to being transcriptionally active, appear to self-process efficiently in the cytoplasm of OST7-1 cells. In particular, the HH and HP(GC) constructs self-process to greater than 95%. The overall extent of self-processing in OST7-1 cells appears to be strikingly similar to the extent of self-processing *in vitro* (Figure 29 "In Vitro +MgCl₂" vs. "Cellular").

Consistent with the *in vitro* self-processing results, the HP(GU) cassette self-processed to approximately 50% in OST7-1 cells. As expected, transfection with plasmids containing the HH(mutant) cassette yielded a primer-extension product corresponding to the full-length RNA with no detectable cleavage products (Figure 29). The latter result strongly suggests that the primer extension band corresponding to the 3' cleavage product is not an artifact of reverse transcription.

Applicant was concerned with the possibility that RNA self-processing might occur during cell lysis, RNA isolation and /or the primer extension assay. Two precautions were taken to exclude this possibility. First, 50 mM EDTA was included in the lysis buffer. EDTA is a strong chelator of divalent

metal ions such as Mg^{2+} and Ca^{2+} that are necessary for ribozyme activity. Divalent metal ions are therefore unavailable to self-processing RNAs following cell lysis. A second precaution involved using primers in the primer-extension assay that were designed to hybridize to essential regions of the processing ribozyme. Binding of these primers should prevent the 3' cis-acting ribozymes from folding into the conformation essential for catalytic activity.

Two experiments were carried out to further eliminate the possibility that self-processing is occurring either during RNA preparations or during the primer extension analysis. The first experiment involves primer extension analysis on full-length precursor RNAs that were added to non-transfected OST7-1 lysates after cell lysis. Thus, only if self-processing is occurring at some point after lysis would cleavage products be detected. Full-length precursor RNAs were prepared by transcribing under conditions of low Mg^{2+} (5 mM) and high NTP concentration (total 12 mM) in an attempt to eliminate the free Mg^{2+} required for the self-processing reaction (Michel et al. 1992 *Genes & Dev.* 6, 1373). The full-length precursor RNAs were gel-purified, and a known amount was added to lysates of non-transfected OST7-1 cells. RNA was purified from these lysates and incubated for 1 hr in DEPC-treated water at 37° C prior to the standard primer extension analysis (Figure 29, *in vitro* "-MgCl₂" control). The predominant RNA detected in all cases corresponds to the primer extension product of full-length precursor RNAs. If, instead, the purified RNA containing the full-length precursor is incubated in 10 mM MgCl₂ prior to the primer extension analysis, most or all of the RNA detected by primer extension analysis undergoes cleavage (Figure 29, *in vitro* "+MgCl₂" control). These results indicate that the standard RNA isolation and primer extension protocols used here do not provide a favorable environment for RNA self-processing, even though the RNA in question is inherently able to undergo self-cleavage.

In a second experiment to demonstrate lack of self-processing during work up, internally-labeled precursor RNAs were prepared and added to non-transfected OST7-1 lysates as in the previous control. The internally-labeled precursor RNAs were carried through the RNA purification and primer extension reactions (in the presence of unlabeled primers) and analyzed to determine the extent of self-processing. By this analysis, the

vast majority of the added full-length RNA remained intact during the entire process of RNA isolation and primer extension.

These two control experiments validate the protocols used and support applicant's conclusion that the self-processing reactions catalyzed
5 by HH, HDV and HP(GC) cassettes are occurring in the cytoplasm of OST7-1 cells.

Sequences in figures 23 through 25 are meant to be non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art.

10 In addition, those in the art will recognize that Applicant provides guidance through the above examples as to how to best design vectors of this invention so that secondary structure of the mRNA allows efficient cleavage by releasing ribozymes. Thus, the specific constructs are not limiting in this invention. Such constructs can be readily tested as
15 described above for such secondary structure, either by computer folding algorithms or empirically. Such constructs will then allow at least 80% completion of release of ribozymes, which can be readily determined as described above or by methods known in the art. That is, any such secondary structure in the RNA does not reduce release of the ribozymes
20 by more than 20%.

IV. Ribozymes Expressed by RNA Polymerase III

Applicant has determined that the level of production of a foreign RNA, using a RNA polymerase III (pol III) based system, can be significantly enhanced by ensuring that the RNA is produced with the 5' terminus and a
25 3' region of the RNA molecule base-paired together to form a stable intramolecular stem structure. This stem structure is formed by hydrogen bond interactions (either Watson-Crick or non-Watson-Crick) between nucleotides in the 3' region (at least 8 bases) and complementary nucleotides in the 5' terminus of the same RNA molecule.

30 Although the example provided below involves a type 2 pol III gene unit, a number of other pol III promoter systems can also be used, for example, tRNA (Hall et al., 1982 *Cell* 29, 3-5), 5S RNA (Nielsen et al., 1993, *Nucleic Acids Res.* 21, 3631-3636), adenovirus VA RNA (Fowlkes and Shenk, 1980 *Cell* 22, 405-413), U6 snRNA (Gupta and Reddy, 1990

Nucleic Acids Res. 19, 2073-2075), vault RNA (Kickoefer et al., 1993 *J. Biol. Chem.* 268, 7868-7873), telomerase RNA (Romero and Blackburn, 1991 *Cell* 67, 343-353), and others.

5 The construct described in this invention is able to accumulate RNA to a significantly higher level than other constructs, even those in which 5' and 3' ends are involved in hairpin loops. Using such a construct the level of expression of a foreign RNA can be increased to between 20,000 and 50,000 copies per cell. This makes such constructs, and the vectors encoding such constructs, excellent for use in decoy, therapeutic editing
10 and antisense protocols as well as for ribozyme formation. In addition, the molecules can be used as agonist or antagonist RNAs (affinity RNAs). Generally, applicant believes that the intramolecular base-paired interaction between the 5' terminus and the 3' region of the RNA should be in a double-stranded structure in order to achieve enhanced RNA
15 accumulation.

Thus, in one preferred embodiment the invention features a pol III promoter system (e.g., a type 2 system) used to synthesize a chimeric RNA molecule which includes tRNA sequences and a desired RNA (e.g., a tRNA-based molecule).

20 The following exemplifies this invention with a type 2 pol III promoter and a tRNA gene. Specifically to illustrate the broad invention, the RNA molecule in the following example has an A box and a B box of the type 2 pol III promoter system and has a 5' terminus or region able to base-pair with at least 8 bases of a complementary 3' end or region of the same RNA
25 molecule. This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using other pol III promoter systems and techniques generally known in the art.

30 By "terminus" is meant the terminal bases of an RNA molecule, ending in a 3' hydroxyl or 5' phosphate or 5' cap moiety. By "region" is meant a stretch of bases 5' or 3' from the terminus that are involved in base-paired interactions. It need not be adjacent to the end of the RNA. Applicant has determined that base pairing of at least one end of the RNA molecule with a region not more than about 50 bases, and preferably only 20 bases, from

the other end of the molecule provides a useful molecule able to be expressed at high levels.

By "3' region" is meant a stretch of bases 3' from the terminus that are involved in intramolecular base-paired interaction with complementary nucleotides in the 5' terminus of the same molecule. The 3' region can be designed to include the 3' terminus. The 3' region therefore is ≥ 0 nucleotides from the 3' terminus. For example, in the S35 construct described in the present invention (Fig. 40) the 3' region is one nucleotide from the 3' terminus. In another example, the 3' region is ~ 43 nt from 3' terminus. These examples are not meant to be limiting. Those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. Generally, it is preferred to have the 3' region within 100 bases of the 3' terminus.

By "tRNA molecule" is meant a type 2 pol III driven RNA molecule that is generally derived from any recognized tRNA gene. Those in the art will recognize that DNA encoding such molecules is readily available and can be modified as desired to alter one or more bases within the DNA encoding the RNA molecule and/or the promoter system. Generally, but not always, such molecules include an A box and a B box that consist of sequences which are well known in the art (and examples of which can be found throughout the literature). These A and B boxes have a certain consensus sequence which is essential for a optimal pol III transcription.

By "chimeric tRNA molecule" is meant a RNA molecule that includes a pol III promoter (type 2) region. A chimeric tRNA molecule, for example, might contain an intramolecular base-paired structure between the 3' region and complementary 5' terminus of the molecule, and includes a foreign RNA sequence at any location within the molecule which does not affect the activity of the type 2 pol III promoter boxes. Thus, such a foreign RNA may be provided at the 3' end of the B box, or may be provided in between the A and the B box, with the B box moved to an appropriate location either within the foreign RNA or another location such that it is effective to provide pol III transcription. In one example, the RNA molecule may include a hammerhead ribozyme with the B box of a type 2 pol III promoter provided in stem II of the ribozyme. In a second example, the B box may be provided in stem IV region of a hairpin ribozyme. A specific example of such RNA molecules is provided below. Those in the art will

recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

By "desired RNA" molecule is meant any foreign RNA molecule which is useful from a therapeutic, diagnostic, or other viewpoint. Such
5 molecules include antisense RNA molecules, decoy RNA molecules, enzymatic RNA, therapeutic editing RNA and agonist and antagonist RNA.

By "antisense RNA" is meant a non-enzymatic RNA molecule that binds to another RNA (target RNA) by means of RNA-RNA interactions and alters the activity of the target RNA (Eguchi et al., 1991 *Annu. Rev. Biochem.* 60, 631-652). By "enzymatic RNA" is meant an RNA molecule
10 with enzymatic activity (Cech, 1988 *J. American. Med. Assoc.* 260, 3030-3035). Enzymatic nucleic acids (ribozymes) act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic
15 portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA.

By "decoy RNA" is meant an RNA molecule that mimics the natural
20 binding domain for a ligand. The decoy RNA therefore competes with natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently binds HIV *tat* protein, thereby preventing it from binding to TAR sequences encoded in the HIV
25 RNA (Sullenger et al., 1990 *Cell* 63, 601-608). This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

By "therapeutic editing RNA" is meant an antisense RNA that can bind
30 to its cellular target (RNA or DNA) and mediate the modification of a specific base.

By "agonist RNA" is meant an RNA molecule that can bind to protein receptors with high affinity and cause the stimulation of specific cellular pathways.

By "antagonist RNA" is meant an RNA molecule that can bind to cellular proteins and prevent it from performing its normal biological function (for example, see Tsai et al., 1992 *Proc. Natl. Acad. Sci. USA* 89, 8864-8868).

5 In other aspects, the invention includes vectors encoding RNA molecules as described above, cells including such vectors, methods for producing the desired RNA, and use of the vectors and cells to produce this RNA.

10 Thus, the invention features a transcribed non-naturally occurring RNA molecule which includes a desired therapeutic RNA portion and an intramolecular stem formed by base-pairing interactions between a 3' region and complementary nucleotides at the 5' terminus in the RNA. The stem preferably includes at least 8 base pairs, but may have more, for example, 15 or 16 base pairs.

15 In preferred embodiments, the 5' terminus of the chimeric tRNA includes a portion of the precursor molecule of the primary tRNA molecule, of which ≥ 8 nucleotides are involved in base-pairing interaction with the 3' region; the chimeric tRNA contains A and B boxes; natural sequences 3' of the B box are deleted, which prevents endogenous RNA processing; the
20 desired RNA molecule is at the 3' end of the B box; the desired RNA molecule is between the A and the B box; the desired RNA molecule includes the B box; the desired RNA molecule is selected from the group consisting of antisense RNA, decoy RNA, therapeutic editing RNA, enzymatic RNA, agonist RNA and antagonist RNA; the molecule has an
25 intramolecular stem resulting from a base-paired interaction between the 5' terminus of the RNA and a complementary 3' region within the same RNA, and includes at least 8 bases; and the 5' terminus is able to base pair with at least 15 bases of the 3' region.

30 In most preferred embodiments, the molecule is transcribed by a RNA polymerase III based promoter system, e.g., a type 2 pol III promoter system; the molecule is a chimeric tRNA, and may have the A and B boxes of a type 2 pol III promoter separated by between 0 and 300 bases; DNA vector encoding the RNA molecule of claim 51.

In other related aspects, the invention features an RNA or DNA vector encoding the above RNA molecule, with the portions of the vector encoding the RNA functioning as a RNA pol III promoter; or a cell containing the vector ; or a method to provide a desired RNA molecule in a cell, by
5 introducing the molecule into a cell with an RNA molecule as described above. The cells can be derived from animals, plants or human beings.

In order for RNA-based gene therapy approaches to be effective, sufficient amounts of the therapeutic RNA must accumulate in the appropriate intracellular compartment of the treated cells. Accumulation is
10 a function of both promoter strength of the antiviral gene, and the intracellular stability of the antiviral RNA. Both RNA polymerase II (pol II) and RNA polymerase III (pol III) based expression systems have been used to produce therapeutic RNAs in cells (Sarver & Rossi, 1993 *AIDS Res. & Human Retroviruses* 9, 483-487; Yu et al., 1993 *P.N.A.S.(USA)* 90, 6340-
15 6344). However, pol III based expression cassettes are theoretically more attractive for use in expressing antiviral RNAs for the following reasons. Pol II produces messenger RNAs located exclusively in the cytoplasm, whereas pol III produces functional RNAs found in both the nucleus and the cytoplasm. Pol II promoters tend to be more tissue restricted, whereas pol
20 III genes encode tRNAs and other functional RNAs necessary for basic "housekeeping" functions in all cell types. Therefore, pol III promoters are likely to be expressed in all tissue types. Finally, pol III transcripts from a given gene accumulate to much greater levels in cells relative to pol II genes.

25 Intracellular accumulation of therapeutic RNAs is also dependent on the method of gene transfer used. For example, the retroviral vectors presently used to accomplish stable gene transfer, integrate randomly into the genome of target cells. This random integration leads to varied expression of the transferred gene in individual cells comprising the bulk
30 treated cell population. Therefore, for maximum effectiveness, the transferred gene must have the capacity to express therapeutic amounts of the antiviral RNA in the entire treated cell population, regardless of the integration site.

Pol III System

The following is just one non-limiting example of the invention. A pol III based genetic element derived from a human tRNA^{met} gene and termed $\Delta 3-5$ (Fig. 33; Adeniyi-Jones et al., 1984 *supra*), has been adapted to express antiviral RNAs (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523). This element was inserted into the DC retroviral vector (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523) to accomplish stable gene transfer, and used to express antisense RNAs against moloney murine leukemia virus and anti-HIV decoy RNAs (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523; Sullenger et al., 1990 *Cell* 63, 601-608; Sullenger et al., 1991 *J. Virol.* 65, 6811-6816; Lee et al., 1992 *The New Biologist* 4, 66-74). Clonal lines are expanded from individual cells present in the bulk population, and therefore express similar amounts of the therapeutic RNA in all cells. Development of a vector system that generates therapeutic levels of therapeutic RNA in all treated cells would represent a significant advancement in RNA based gene therapy modalities.

Applicant examined hammerhead (HHI) ribozyme (RNA with enzymatic activity) expression in human T cell lines using the $\Delta 3-5$ vector system (These constructs are termed " $\Delta 3-5$ /HHI"; Fig. 34). On average, ribozymes were found to accumulate to less than 100 copies per cell in the bulk T cell populations. In an attempt to improve expression levels of the $\Delta 3-5$ chimera, the applicant made a series of modified $\Delta 3-5$ gene units containing enhanced promoter elements to increase transcription rates, and inserted structural elements to improve the intracellular stability of the ribozyme transcripts (Fig. 34). One of these modified gene units, termed S35, gave rise to more than a 100-fold increase in ribozyme accumulation in bulk T cell populations relative to the original $\Delta 3-5$ /HHI vector system. Ribozyme accumulation in individual clonal lines from the pooled T cell populations ranged from 10 to greater than 100 fold more than those achieved with the original $\Delta 3-5$ /HHI version of this vector.

The S35 gene unit may be used to express other therapeutic RNAs including, but not limited to, ribozymes, antisense, decoy, therapeutic editing, agonist and antagonist RNAs. Application of the S35 gene unit would not be limited to antiviral therapies, but also to other diseases, such as cancer, in which therapeutic RNAs may be effective. The S35 gene unit may be used in the context of other vector systems besides retroviral

vectors, including but not limited to, other stable gene transfer systems such as adeno-associated virus (AAV; Carter, 1992 *Curr. Opin. Genet. Dev.* 3, 74), as well as transient vector systems such as plasmid delivery and adenoviral vectors (Berkner, 1988 *BioTechniques* 6, 616-629).

5 As described below, the S35 vector encodes a truncated version of a tRNA wherein the 3' region of the RNA is base-paired to complementary nucleotides at the 5' terminus, which includes the 5' precursor portion that is normally processed off during tRNA maturation. Without being bound by any theory, Applicant believes this feature is important in the level of
10 expression observed. Thus, those in the art can now design equivalent RNA molecules with such high expression levels. Below are provided examples of the methodology by which such vectors and tRNA molecules can be made.

Δ3-5 Vectors

15 The use of a truncated human tRNA^{met} gene, termed Δ3-5 (Fig. 33; Adeniyi-Jones et al., 1984 *supra*), to drive expression of antisense RNAs, and subsequently decoy RNAs (Sullenger et al., 1990 *supra*) has recently been reported. Because tRNA genes utilize internal pol III promoters, the antisense and decoy RNA sequences were expressed as chimeras
20 containing tRNA^{met} sequences. The truncated tRNA genes were placed into the U3 region of the 3' moloney murine leukemia virus vector LTR (Sullenger et al., 1990 *supra*).

Base-Paired Structures

Since the Δ3-5 vector combination has been successfully used to
25 express inhibitory levels of both antisense and decoy RNAs, applicant cloned ribozyme-encoding sequences (termed as "Δ3-5/HHI") into this vector to explore its utility for expressing therapeutic ribozymes. However, low ribozyme accumulation in human T cell lines stably transduced with this vector was observed (Fig. 35). To try and improve accumulation of the
30 ribozyme, applicant incorporated various RNA structural elements (Fig. 34) into one of the ribozyme chimeras (Δ3-5/HHI).

Two strategies were used to try and protect the termini of the chimeric transcripts from exonucleolytic degradation. One strategy involved the incorporation of stem-loop structures into the termini of the transcript. Two

such constructs were cloned, S3 which contains a stem-loop structure at the 3' end, and S5 which contains stem-loop structures at both ends of the transcript (Figure 34). The second strategy involved modification of the 3' terminal sequences such that the 5' terminus and the 3' end sequences
5 can form a stable base-paired stem. Two such constructs were made: S35 in which the 3' end was altered to hybridize to the 5' leader and acceptor stem of the tRNA^{met} domain, and S35Plus which was identical to S35 but included more extensive structure formation within the non-ribozyme portion of the $\Delta 3-5$ chimeras (Figure 34). These stem-loop structures are
10 also intended to sequester non-ribozyme sequences in structures that will prevent them from interfering with the catalytic activity of the ribozyme. These constructs were cloned, producer cell lines were generated, and stably-transduced human MT2 (Harada et al., 1985 *supra*) and CEM (Nara & Fischinger, 1988 *supra*) cell lines were established (*Curr. Protocols Mol.*
15 *Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). The RNA sequences and structure of S35 and S35 Plus are provided in Figures 40-47.

Referring to Figure 48, there is provided a general structure for a chimeric RNA molecule of this invention. Each N independently represents none or a number of bases which may or may not be base paired. The A
20 and B boxes are optional and can be any known A or B box, or a consensus sequence as exemplified in the figure. The desired nucleic acid to be expressed can be any location in the molecule, but preferably is on those places shown adjacent to or between the A and B boxes (designated by arrows). Figure 49 shows one example of such a structure in which a
25 desired RNA is provided 3' of the intramolecular stem. A specific example of such a construct is provided in Figures 50 and 51.

Example 26: Cloning of $\Delta 3-5$ -Ribozyme Chimera

Oligonucleotides encoding the S35 insert that overlap by at least 15 nucleotides were designed (5' GATCCACTCTGCTGTTCTGTTTTGA 3'
30 and 5' CGCGTCAAAAACAGAACAGCAGAGTG 3'). The oligonucleotides (10 μ M each) were denatured by boiling for 5 min in a buffer containing 40 mM Tris.HCl, pH8.0. The oligonucleotides were allowed to anneal by snap cooling on ice for 10-15 min.

The annealed oligonucleotide mixture was converted into a double-
35 stranded molecule using Sequenase[®] enzyme (US Biochemicals) in a

buffer containing 40 mM Tris.HCl, pH7.5, 20 mM MgCl₂, 50 mM NaCl, 0.5 mM each of the four deoxyribonucleotide triphosphates, 10 mM DTT. The reaction was allowed to proceed at 37°C for 30 min. The reaction was stopped by heating to 70°C for 15 min.

- 5 The double stranded DNA was digested with appropriate restriction endonucleases (*Bam*HI and *Mlu*I) to generate ends that were suitable for cloning into the Δ 3-5 vector.

- 10 The double-stranded insert DNA was ligated to the Δ 3-5 vector DNA by incubating at room temperature (about 20°C) for 60 min in a buffer containing 66 mM Tris.HCl, pH 7.6, 6.6 mM MgCl₂, 10 mM DTT, 0.066 μ M ATP and 0.1U/ μ l T4 DNA Ligase (US Biochemicals).

- 15 Competent *E. coli* bacterial strain was transformed with the recombinant vector DNA by mixing the cells and DNA on ice for 60 min. The mixture was heat-shocked by heating to 37°C for 1 min. The reaction mixture was diluted with LB media and the cells were allowed to recover for 60 min at 37°C. The cells were plated on LB agar plates and incubated at 37°C for ~ 18 h.

- 20 Plasmid DNA was isolated from an overnight culture of recombinant clones using standard protocols (Ausubel et al., *Curr. Protocols Mol. Biology* 1990, Wiley & Sons, NY).

The identity of the clones were determined by sequencing the plasmid DNA using the Sequenase[®] DNA sequencing kit (US Biochemicals).

- 25 The resulting recombinant Δ 3-5 vector contains the S35 sequence. The HHI encoding DNA was cloned into this Δ 3-5-S35 containing vector using *Sac*II and *Bam*HI restriction sites.

Example 27: Northern analysis

- 30 RNA from the transduced MT2 cells were extracted and the presence of Δ 3-5/ribozyme chimeric transcripts were assayed by Northern analysis (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). Northern analysis of RNA extracted from MT2 transductants showed that Δ 3-5/ribozyme chimeras of appropriate sizes were expressed (Fig. 35,36). In addition, these results demonstrated the relative differences in accumulation among the different constructs (Figure 35,36). The pattern of

expression seen from the $\Delta 3$ -5/HHI ribozyme chimera was similar to 12 other ribozymes cloned into the $\Delta 3$ -5 vector (not shown). In MT-2 cell line, $\Delta 3$ -5/HHI ribozyme chimeras accumulated, on average, to less than 100 copies per cell.

- 5 Addition of a stem-loop onto the 3' end of $\Delta 3$ -5/HHI did not lead to increased $\Delta 3$ -5 levels (S3 in Fig. 35.36). The S5 construct containing both 5' and 3' stem-loop structures also did not lead to increased ribozyme levels (Fig. 35.36).

- 10 Interestingly, the S35 construct expression in MT2 cells was about 100-fold more abundant relative to the original $\Delta 3$ -5/HHI vector transcripts (Fig. 35.36). This may be due to increased stability of the S35 transcript.

Example 28: Cleavage activity

- 15 To assay whether ribozymes transcribed in the transduced cells contained cleavage activity, total RNA extracted from the transduced MT2 T cells were incubated with a labeled substrate containing the HHI cleavage site (Figure 37). Ribozyme activity in all but the S35 constructs, was too low to detect. However, ribozyme activity was detectable in S35-transduced T cell RNA. Comparison of the activity observed in the S35-transduced MT2 RNA with that seen with MT2 RNA in which varying
20 amounts of in vitro transcribed S5 ribozyme chimeras, indicated that between 1-3 nM of S35 ribozyme was present in S35-transduced MT2 RNA. This level of activity corresponds to an intracellular concentration of 5,000-15,000 ribozyme molecules per cell.

Example 29: Clonal variation

- 25 Variation in the ribozyme expression levels among cells making up the bulk population was determined by generating several clonal cell lines from the bulk S35 transduced CEM line (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY) and the ribozyme expression and activity levels in the individual clones were measured (Figure 38 and 39).
30 All the individual clones were found to express active ribozyme. The ribozyme activity detected from each clone correlated well with the relative amounts of ribozyme observed by Northern analysis. Steady state ribozyme levels among the clones ranged from approximately 1,000 molecules per cell in clone G to 11,000 molecules per cell in clone H (Fig.

38). The mean accumulation among the clones, calculated by averaging the ribozyme levels of the clones, exactly equaled the level measured in the parent bulk population. This suggests that the individual clones are representative of the variation present in the bulk population.

- 5 The fact that all 14 clones were found to express ribozyme indicate that the percentage of cells in the bulk population expressing ribozyme is also very high. In addition, the lowest level of expression in the clones was still more than 10-fold that seen in bulk cells transduced with the original $\Delta 3-5$ vector. Therefore, the S35 gene unit should be much more effective
10 in a gene therapy setting in which bulk cells are removed, transduced and then reintroduced back into a patient.

Example 30: Stability

- Finally, the bulk S35-transduced line, resistant to G418, was propagated for a period of 3 months (in the absence of G418) to determine
15 If ribozyme expression was stable over extended periods of time. This situation mimicks that found in the clinic in which bulk cells are transduced and then reintroduced into the patient and allowed to propagate. There was a modest 30% reduction of ribozyme expression after 3 months. This difference probably arose from cells with varying amount of ribozyme
20 expression and exhibiting different growth rates in the culture becoming slightly more prevalent in the culture. However, ribozyme expression is apparently stable for at least this period of time.

Example 31: Design and construction of TRZ-tRNA Chimera

- A transcription unit, termed TRZ, is designed that contains the S35
25 motif (Figure 52). A desired RNA (e.g. ribozyme) can be inserted into the indicated region of TRZ tRNA chimera. This construct might provide additional stability to the desired RNA. TRZ-A and TRZ-B are non-limiting examples of the TRZ-tRNA chimera.

- Referring to Fig. 53-54, a hammerhead ribozyme targeted to site I
30 (HHITRZ-A; Fig. 53) and a hairpin ribozyme (HPITRZ-A; Fig. 54), also targeted to site I, is cloned individually into the indicated region of TRZ tRNA chimera. The resulting ribozyme transcripts retain full RNA cleavage activity (see for example Fig. 55). Applicant has shown that efficient

expression of these TRZ tRNA chimera can be achieved in mammalian cells.

Besides ribozymes, desired RNAs like antisense, therapeutic editing RNAs, decoys, can be readily inserted into the indicated region of TRZ-tRNA chimera to achieve therapeutic levels of RNA expression in mammalian cells.

Sequences listed in Figures 40-47 and 50 - 54 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (mutations, insertions and deletions) of the above examples can be readily generated using techniques known in the art, are within the scope of the present invention.

Example 32: Ribozyme expression in T cell lines

Ribozyme expression in T cell lines stably-transduced with either a retroviral-based or an Adeno-associated virus (AAV)-based ribozyme expression vector (Figure 56). The human T cell lines MT2 and CEM were transduced with either retroviral or AAV vectors encoding a neomycin selectable marker and a ribozyme (S35/HHI) expressed from pol III met_i tRNA-driven promoter. Cells stably-transduced with the vectors were selectively expanded in medium containing the neomycin antibiotic derivative, G418 (0.7 mg/ml). Ribozyme expression in the stable cell lines was then analyzed by Northern analysis. The probe used to detect ribozyme transcripts also cross-hybridized with human met_i tRNA sequences. Referring to Figure 56, S35/HHI RNA accumulates to significant levels in MT2 and CEM cells when transduced with either the retrovirus or the AAV vector.

These are meant to be non-limiting examples, those skilled in the art will recognize that other vectors such as adenovirus vector (Figure 57), plasmid DNA vector, alpha virus vectors and the other derivatives thereof, can be readily generated to deliver the desired RNA, using techniques known in the art and are within the scope of this invention. Additionally, the transcription units can be expressed individually or in multiples using pol II and/or pol III promoters.

References cited herein, as well as Draper WO 93/23569, 94/02495, 94/06331, Sullenger WO 93/12657, Thompson WO 93/04573, and Sullivan

WO 94/04609, and 93/11253 describe methods for use of vectors described herein, and are incorporated by reference herein. In particular these vectors are useful for administration of antisense and decoy RNA molecules.

5 Example 33: Ligated Ribozymes are catalytically active

The ability of ribozymes generated by ligation methods, described in Draper et al., PCT WO 93/23569, to cleave target RNA was tested on either matched substrate RNA (Fig. 58) or long (622 nt) RNA (Fig. 59, 60 and 61).

10 Matched substrate RNAs were chemically synthesized using solid-phase RNA synthesis chemistry (Scaringe et al., 1990 Nucleic Acids Res. 18, 5433-5441). Substrate RNA was 5' end-labeled using [γ - 32 P] ATP and polynucleotide kinase (Curr. Protocols Mol. Biol. 1992, ed. Ausubel et al., Wiley & Sons, NY). Ribozyme reactions were carried out under ribozyme excess conditions (k_{cat}/K_M ; Herschlag and Cech, 1990 Biochemistry 29, 15 10159-10171). Briefly, ribozyme and substrate RNA were denatured and renatured separately by heating to 90°C and snap cooling on ice for 10 min in a buffer containing 50 mM Tris. HCl pH 7.5 and 10 mM MgCl₂. Cleavage reaction was initiated by mixing the ribozyme with the substrate at 37°C. Aliquots of 5 μ l were taken at regular intervals of time and the 20 reaction was stopped by mixing with equal volume of formamide gel loading buffer (Curr. Protocols Mol. Biol. 1992, ed. Ausubel et al., Wiley & Sons, NY). The samples were resolved on 20 % polyacrylamide-urea gel. Referring to Fig. 58, $-\Delta G$ refers to the free energy of binding calculated for base-paired interactions between the ribozyme and the substrate RNA 25 (Turner and Sugimoto, 1988 Supra). RPI A is a HH ribozyme with 6/6 binding arms. This ribozyme was synthesized chemically either as a one piece ribozyme or was synthesized in two fragments followed by ligation to generate a one piece ribozyme. The k_{cat}/K_M values for the two ribozymes were comparable.

30 A template containing T7 RNA polymerase promoter upstream of 622 nt long target sequence, was PCR amplified from a DNA clone. The target RNA (containing HH ribozyme cleavage sites B, C and D) was transcribed from this PCR amplified template using T7 RNA polymerase. The transcript was internally labeled during transcription by including [α - 32 P] CTP as one 35 of the four ribonucleotide triphosphates. The transcription mixture was

treated with DNase-1, following transcription at 37°C for 2 hours, to digest away the DNA template used in the transcription. RNA was precipitated with isopropanol and the pellet was washed two times with 70% ethanol to get rid of salt and nucleotides used in the transcription reaction. RNA is resuspended in DEPC-treated water and stored at 4°C. Ribozyme cleavage reactions were carried out under ribozyme excess (k_{cat}/K_M) conditions [Herschlag and Cech 1990 *supra*]. Briefly, 1000 nM ribozyme and 10 nM internally labeled target RNA were denatured separately by heating to 90°C for 2 min in the presence of 50 mM Tris.HCl, pH 7.5 and 10 mM MgCl₂. The RNAs were renatured by cooling to 37°C for 10-20 min. Cleavage reaction was initiated by mixing the ribozyme and target RNA at 37°C. Aliquots of 5 µl were taken at regular intervals of time and the reaction was quenched by adding equal volume of stop buffer. The samples were resolved on a sequencing gel.

15 Example 34: Hammerhead ribozymes with ≥ 2 base-paired stem II are catalytically active

To decrease the cost of chemical synthesis of RNA, applicant was interested in determining whether the length of stem II region of a typical hammerhead ribozyme (≥ 4 bp stem II) can be shortened without decreasing the catalytic efficiency of the HH ribozyme. The length of stem II was systematically shortened by one base-pair at a time. HH ribozymes with three and two base-paired stem II were chemically synthesized using solid-phase RNA phosphoramidite chemistry (Scaringe *et al.*, 1990 *supra*).

Matched and long substrate RNAs were synthesized and ribozyme assays were carried out as described in example 33. Referring to figures 62, 63 and 64, data shows that shortening stem II of a hammerhead ribozyme does not significantly alter the catalytic efficiency. It is applicant's opinion that hammerhead ribozymes with ≥ 2 base-paired stem II region are catalytically active.

30 Example 35: Synthesis of catalytically active hairpin ribozymes

RNA molecules were chemically synthesized having the nucleotide base sequence shown in Fig. 65 for both the 5' and 3' fragments. The 3' fragments are phosphorylated and ligated to the 5' fragment essentially as described in example 37. As is evident from the Figure 65, the 3' and 5' fragments can hybridize together at helix 4 and are covalently linked via

GAAA sequence. When this structure hybridizes to a substrate, a ribozyme-substrate complex structure is formed. While helix 4 is shown as 3 base pairs it may be formed with only 1 or 2 base pairs.

40 nM mixtures of ligated ribozymes were incubated with 1-5 nM 5' end-labeled matched substrates (chemically synthesized by solid-phase synthesis using RNA phosphoramidite chemistry) for different times in 50 mM Tris/HCl pH 7.5, 10 mM MgCl₂ and shown to cleave the substrate efficiently (Fig.66).

The target and the ribozyme sequences shown in Fig. 62 and 65 are meant to be non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using other sequences and techniques generally known in the art.

V. Constructs of Hairpin Ribozymes

There follows an improved trans-cleaving hairpin ribozyme in which a new helix (*i.e.*, a sequence able to form a double-stranded region with another single-stranded nucleic acid) is provided in the ribozyme to base-pair with a 5' region of a separate substrate nucleic acid. This helix is provided at the 3' end of the ribozyme after helix 3 as shown in Figure 3. In addition, at least two extra bases may be provided in helix 2 and a portion of the substrate corresponding to helix 2 may be either directly linked to the 5' portion able to hydrogen bond to the 3' end of the hairpin or may have a linker of at least one base. By trans-cleaving is meant that the ribozyme is able to act in *trans* to cleave another RNA molecule which is not covalently linked to the ribozyme itself. Thus, the ribozyme is not able to act on itself in an intramolecular cleavage reaction.

By "base-pair" is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example Hoogsteen type) of interactions.

The increase in length of helix 2 of a hairpin ribozyme (with or without helix 5) has several advantages. These include improved stability of the ribozyme-target complex *in vivo*. In addition, an increase in the recognition sequence of the hairpin ribozyme improves the specificity of the ribozyme. This also makes possible the targeting of potential hairpin

ribozyme sites that would otherwise be inaccessible due to neighboring secondary structure.

The increase in length of helix 2 of a hairpin ribozyme (with or without helix 5) enhances *trans*-ligation reaction catalyzed by the ribozyme. *Trans*-ligation reactions catalyzed by the regular hairpin ribozyme (4 bp helix 2) is very inefficient (Komatsu *et al.*, 1993 *Nucleic Acids Res.* 21, 185). This is attributed to weak base-pairing interactions between substrate RNAs and the ribozyme. By increasing the length of helix 2 (with or without helix 5) the rate of ligation (*in vitro* and *in vivo*) can be enhanced several fold.

- 10 Results of experiments suggest that the length of H2 can be 6 bp without significantly reducing the activity of the hairpin ribozyme. The H2 arm length variation does not appear to be sequence dependent. HP ribozymes with 6 bp H2 have been designed against five different target RNAs and all five ribozymes efficiently cleaved their cognate target RNA.
- 15 Additionally, two of these ribozymes were able to successfully inhibit gene expression (e.g., TNF- α) in mammalian cells. Results of these experiments are shown below.

- HP ribozymes with 7 and 8 bp H2 are also capable of cleaving target RNA in a sequence-specific manner, however, the rate of the cleavage reaction is lower than those catalyzed by HP ribozymes with 6 bp H2.
- 20

Example 36: 4 and 6 base pair H2

Referring to Figures 67-72, HP ribozymes were synthesized as described above and tested for activity. Surprisingly, those with 6 base pairs in H2 were still as active as those with 4 base pairs.

25 VI. Chemical Modification

Oligonucleotides with 5'-C-alkyl Group

- The introduction of an alkyl group at the 5'-position of a nucleoside or nucleotide sugar introduces an additional center of chirality into the sugar moiety. Referring to Fig. 75, the general structures of 5'-C-alkylnucleotides belonging to the D-allose, 2, and L-talose, 3, sugar families are shown.
- 30 The family names are derived from the known sugars D-allose and L-talose ($R_1 = CH_3$ in 2 and 3 in Figure 75). Useful specific D-allose and L-talose

nucleotide derivatives are shown in Figure 76, 29-32 and Figure 77, 58-61 respectively.

This invention relates to the use of 5'-C-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 5'-C-alkylnucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 5'-C-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 5'-C-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Such nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 5'-C-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 5'-C-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

Thus, in one aspect, the invention features 5'-C-alkylnucleosides, that is a nucleotide base having at the 5'-position on the sugar molecule an alkyl moiety. In a related aspect, the invention also features 5'-C-alkylnucleotides, and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably

includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above. In preferred embodiments, the sugar of the nucleoside or nucleotide is in an optically pure form, as the talose or allose sugar.

5 Examples of various alkyl groups useful in this invention are shown in Figure 75, where each R_1 group is any alkyl. These examples are not limiting in the invention. Specifically, an "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More
10 preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $\text{N}(\text{CH}_3)_2$, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one
15 carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy,
20 =O, =S, NO_2 , halogen, $\text{N}(\text{CH}_3)_2$, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons,
25 more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $\text{N}(\text{CH}_3)_2$, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an
30 aromatic group which has at least one ring having a conjugated π electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an
35 alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring

atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

10 In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 5'-C-alkylnucleotides; *e.g.* enzymatic nucleic acids having a 5'-C-alkylnucleotide; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic
15 molecule with at least one nucleotide having at its 5'-position an alkyl group. In other related aspects, the invention features 5'-C-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

20 The 5'-C-alkyl derivatives of this invention provide enhanced stability to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall activity *in vivo*. Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

25 In another aspect, the invention features a method for conversion of a protected allo sugar to a protected talo sugar. In the method, the protected allo sugar is contacted with triphenyl phosphine, diethylazodicarboxylate, and *p*-nitrobenzoic acid under inversion causing conditions to provide the protected talo sugar. While one example of such conditions is provided
30 below, those in the art will recognize other such conditions. Applicant has found that such conversion allows for ready synthesis of all types of nucleotide bases as exemplified in the figures.

While this invention is applicable to all oligonucleotides, applicant has found that the modified molecules of this invention are particularly useful for
35 enzymatic RNA molecules. Thus, below is provided examples of such

molecules. Those in the art will recognize that equivalent procedures can be used to make other molecules without such enzymatic activity. Specifically, Figure 1 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. This is not to be taken as an indication that the Figure is prior art to the pending claims, or that the art discussed is prior art to those claims. Referring to Figure 1, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 5'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Substitutions of any nucleotide with any of the modified nucleotides shown in Figure 75 are possible.

The following are non-limiting examples showing the synthesis of nucleic acids using 5'-C-alkyl-substituted phosphoramidites and the syntheses of the amidites.

Example 37: Synthesis of Hammerhead Ribozymes Containing 5'-C-Alkyl-nucleotides & Other Modified Nucleotides

The method of synthesis would follow the procedure for normal RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 26-29 and 56-59). These 5'-C-alkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group 1 or Group 2 intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 38: Methyl-2,3-O-Isopropylidene-6-Deoxy-β-D-allofuranoside (4)

A suspension of L-rhamnose (100 g, 0.55 mol), CuSO₄ (120 g) and conc. H₂SO₄ (4.0 mL) in 1.0 L of dry acetone was mixed for 24 h at RT, then filtered. Conc. NH₄OH (5 mL) was added to the filtrate and the newly formed precipitate was filtered. The residue was concentrated *in vacuo*, coevaporated with pyridine (2 x 300 mL), dissolved in pyridine (500 mL) and cooled to 0 °C. A solution of *p*-toluenesulfonylchloride (107 g , 0.56

mmol) in dry DCE (500 mL) was added dropwise over 0.5 h. The reaction mixture was left for 16 h at RT. The reaction was quenched by adding ice-water (0.5 L) and, after mixing for 0.5 h, was extracted with chloroform (0.75 L). The organic layer was washed with H₂O (2 x 500 mL), 10% H₂SO₄ (2 x 300 mL), water (2 x 300 mL), sat. NaHCO₃ (2 x 300 mL), brine (2 x 300 mL), dried over MgSO₄ and evaporated to dryness. The residue (115 g) was dissolved in dry MeOH (1 L) and treated with NaOMe (23.2 g, 0.42 mmol) in MeOH. The reaction mixture was left for 16 h at 20 °C, neutralized with dry CO₂ and evaporated to dryness. The residue was suspended in chloroform (750 mL), filtered, concentrated to 100 mL and purified by flash chromatography in CHCl₃ to yield 45 g (37%) of compound 4.

Example 39: Methyl-2,3-O-Isopropylidene-5-O-*t*-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (5).

To solution of methylfuranoside 4 (12.5 g 62.2 mmol) and AgNO₃ (21.25 g, 125.0 mmol) in dry DMF (300 mL) *t*-butyldiphenylsilyl chloride (22.2 g, 81 mmol) was added dropwise under Ar over 0.5 h. The reaction mixture was stirred for 4 h at RT, diluted with CHCl₃ (200 mL), filtered and evaporated to dryness (below 40 °C using a high vacuum oil pump). The residue was dissolved in CH₂Cl₂ (300 mL) washed with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL), dried over MgSO₄ and evaporated to dryness. The residue was purified by flash chromatography in CH₂Cl₂ to yield 20.0 g (75%) of compound 5.

Example 40: Methyl-5-O-*t*-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (6).

Methylfuranoside 5 (13.5 g, 30.6 mmol) was dissolved in CF₃COOH:dioxane:H₂O / 2:1:1 (v/v/v, 200 mL) and stirred at 24 °C for 45 m. The reaction mixture was cooled to -10 °C, neutralized with conc. NH₄OH (140 mL) and extracted with CH₂Cl₂ (500 mL). The organic layer was separated, washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL), dried over MgSO₄ and evaporated to dryness. The product 6 was purified by flash chromatography using a 0-10% MeOH gradient in CH₂Cl₂. Yield 9.0 g (76%).

Example 41: Methyl-2,3-di-O-Benzoyl-5-O-t-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (7).

Methylfuranoside 6 (7.0 g, 17.5 mmol) was coevaporated with pyridine (2 x 100 mL) and dissolved in pyridine (100 mL). Benzoyl chloride (5.4 g, 38.5 mmol) was added and the reaction mixture was left at RT for 16 h. Dry EtOH (50 mL) was added and the reaction mixture was evaporated to dryness after 0.5 h. The residue was dissolved in CH₂Cl₂ (300 mL), washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL) dried over MgSO₄ and evaporated to dryness. The product was purified by flash chromatography in CH₂Cl₂ to yield 9.5 g (89%) of compound 7.

Example 42: 1-O-Acetyl-2,3-di-O-benzoyl-5-O-t-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranose (8).

Dibenzoate 7 (4.7 g, 7.7 mmol) was dissolved in a mixture of AcOH (10.0 mL), Ac₂O (20.0 mL) and EtOAc (30 mL) and the reaction mixture was cooled 0 °C. 98% H₂SO₄ (0.15 mL) was then added. The reaction mixture was kept at 0 °C for 16 h, and then poured into a cold 1:1 mixture of sat. NaHCO₃ and EtOAc (150 mL). After 0.5 h of vigorous stirring the organic phase was separated, washed with brine (2 x 75 mL), dried over MgSO₄, evaporated to dryness and coevaporated with toluene (2 x 50 mL). The product was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 4.0 g (82% as a mixture of α and β isomers).

Example 43: 1-(2',3'-di-O-Benzoyl-5'-O-t-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)uracil (9).

Uracil (1.44 g, 11.5 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (3 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT, evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (6.36 g, 10.0 mmol) in dry CH₃CN (100 mL), followed by CF₃SO₃SiMe₃ (2.8 g, 12.6 mmol). The reaction mixture was kept at 24 °C for 16 h, concentrated to 1/3 of its original volume, diluted with 100 mL of CH₂Cl₂ and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄, and evaporated to dryness. The product 9 was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 5.7 g (80%).

Example 44: *N*⁴-Benzoyl-1-(2',3'-Di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)Cytosine (10).

*N*⁴-benzoylcytosine (1.84 g, 8.56 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (3 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of of acetates 8 (3.6 g, 5.6 mmol) in dry CH₃CN (100 mL), followed by CF₃SO₃SiMe₃ (4.76 g, 21.4 mmol). The reaction mixture was boiled under reflux for 5 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. Purification by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ yielded 1.8 g (55%) of compound 10.

Example 45: *N*⁶-Benzoyl-9-(2',3'-di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)adenine (11).

*N*⁶-benzoyladenine (2.86 g, 11.86 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (7 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of of acetates 8 (3.6 g, 5.6 mmol) in dry CH₃CN (100 mL) followed by CF₃SO₃SiMe₃ (6.59 g, 29.7 mmol). The reaction mixture was boiled under reflux for 8 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. The product 11 was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 2.7 g (60%).

Example 46: *N*²-Isobutyryl-9-(2',3'-di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)guanine (12).

*N*²-Isobutyrylguanine (1.47 g, 11.2 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (6 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a

- solution of acetates 8 (3.4 g, 5.3 mmol) in dry CH₃CN (100 mL) followed by CF₃SO₃SiMe₃ (6.22 g, 28.0 mmol). The reaction mixture was boiled under reflux for 8 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL),
5 brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. The product 12 was purified by flash chromatography using a gradient of 0-2% MeOH in CH₂Cl₂. Yield: 2.1g (54%).

Example 47: N⁶-Benzoyl-9-(2',3'-di-O-benzoyl-6'-Deoxy-β-D-Allofuranosyl)adenine (15).

- 10 Nucleoside 11 (1.65 g, 2.0 mmol) was dissolved in THF (50 mL) and a 1 M solution of TBAF in THF (4 mL) was added. The reaction mixture was kept at RT for 4 h, evaporated to dryness and the product purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ to yield 1.0 g (85%) of compound 15.

- 15 Example 48: N⁶-Benzoyl-9-(2',3'-di-O-Benzoyl-5'-O-Dimethoxytrityl-6'-Deoxy-β-D-Allofuranosyl)-adenine (19).

- Nucleoside 15 (0.55 g, 0.92 mmol) was dissolved in dry CH₂Cl₂ (50 mL). AgNO₃ (0.34 g, 2.0 mmol), dimethoxytrityl chloride (0.68 g, 2.0 mmol) and sym-collidine (0.48 g) were added under Ar. The reaction mixture was
20 stirred for 2h, diluted with CH₂Cl₂ (100 mL), filtered, evaporated to dryness and coevaporated with toluene (2 x 50 mL). Purification by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ yielded 0.8 g (97%) of compound 19.

- Example 49: N⁶-Benzoyl-9-(-5'-O-Dimethoxytrityl-6'-Deoxy-β-D-Allo-
25 furanosyl)adenine (23).

- Nucleoside 19 (1.8 g, 2 mmol) was dissolved in dioxane (50 mL), cooled to 0 °C and 2 M NaOH (50 mL) was added. The reaction mixture was kept at 0 °C for 45 m, neutralized with Dowex 50 (Pyr⁺ form), filtered and the resin was washed with MeOH (2 x 50 mL). The filtrate was then
30 evaporated to dryness. Purification by flash chromatography using a gradient of 0-10% MeOH in CH₂Cl₂ yielded 1.1 g (80%) of 23.

Example 50: *N*⁶-Benzoyl-9-(-5'-O-Dimethoxytrityl-2'-O-*t*-butyldimethylsilyl-6'-Deoxy-β-D-Allofuranosyl)adenine (27).

Nucleoside 23 (1.2 g, 1.8 mmol) was dissolved in dry THF (50 mL). Pyridine (0.50 g, 8 mmol) and AgNO₃ (0.4 g, 2.3 mmol) were added. After
5 the AgNO₃ dissolved (1.5 h), *t*-butyldimethylsilyl chloride (0.35 g, 2.3 mmol) was added and the reaction mixture was stirred at RT for 16 h. The reaction mixture was diluted with CH₂Cl₂ (100 mL), filtered into sat. NaHCO₃ (50 mL), extracted, the organic layer washed with brine (2 x 50 mL), dried over MgSO₄ and evaporated to dryness. The product 27 was
10 purified by flash chromatography using a hexanes:EtOAc / 7:3 gradient. Yield: 0.7 g (50%).

Example 51: *N*⁶-Benzoyl-9-(-5'-O-Dimethoxytrityl-2'-O-*t*-butyldimethylsilyl-6'-Deoxy-β-D-Allofuranosyl)adenine-3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (31).

15 Standard phosphitylation of 27 according to Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidite 31 in 73% yield.

Example 52: Methyl-5-O-*p*-Nitrobenzoyl-2,3-O-Isopropylidene-6-deoxy-β-L-Tallofuranoside (5)

20 Methylfuranoside 4 (3.1 g 14.2 mmol) was dissolved in dry dioxane (200 mL), *p*-nitrobenzoic acid (10.0 g, 60 mmol) and triphenylphosphine (15.74 g, 60.0 mmol) were added followed by DEAD (10.45 g, 60.0 mmol). The reaction mixture was left at RT for 16 h, EtOH (5 mL) was added, and after 0.5 h the reaction mixture was evaporated to dryness. The residue
25 was dissolved in CH₂Cl₂ (300 mL) washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL) dried over MgSO₄ and evaporated to dryness. Purification by flash chromatography using a hexanes:EtOAc / 9:1 gradient yielded 4.1 g (78%) of compound 33. Subsequent debenzoylation (NaOMe/MeOH) and silylation (see preparation of 5) led to L-talofuranoside 34 which was converted to phosphoramidites 58-61 using
30 the same methodology as described above for the preparation of the phosphoramidites of the D-allo-isomers 29-32.

The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage

or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan et al., PCT WO 94/ 02595.

- 5 The ribozymes and the target RNA containing site O were synthesized, deprotected and purified as described above. RNA cleavage assay was carried out at 37°C in the presence of 10 mM MgCl₂ as described above.

- 10 Applicant has substituted 5'-C-Me-L-talo nucleotides at positions A6, A9, A9 + G10, C11.1 and C11.1 + G10, as shown in Figure 78 (HH-O1 to HH-O5). HH-O 1,2,4 and 5 showed almost wild type activity (Figure 79). However, HH-O3 demonstrated low catalytic activity. Ribozymes HH-O1, 2, 3, 4 and 5 are also extremely resistant to degradation by human serum nucleases.

15 Oligonucleotides with 2'-Deoxy-2'-Alkyl nucleotide

- 20 This invention uses 2'-deoxy-2'-alkyl nucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 2'-deoxy-2'-alkyl nucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 2'-deoxy-2'-alkyl nucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

- 30 Also within the invention are 2'-deoxy-2'-alkyl nucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Contrary to the findings of De Mesmaeker et al. applicant has found that such nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 2'-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair
- 35

forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 2'-deoxy-2'-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

Thus, in one aspect, the invention features 2'-deoxy-2'-alkylnucleotides, that is a nucleotide base having at the 2'-position on the sugar molecule an alkyl moiety and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

Examples of various alkyl groups useful in this invention are shown in Figure 81, where each R group is any alkyl. The term "alkyl" does not include alkoxy groups which have an "-O-alkyl" group, where "alkyl" is defined as described above, where the O is adjacent the 2'-position of the sugar molecule.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-deoxy-2'-alkylnucleotides (preferably not a 2'-alkyl- uridine or thymidine); e.g. enzymatic nucleic acids having a 2'-deoxy-2'-alkylnucleotide; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group. In other related aspects, the invention features 2'-deoxy-2'-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-alkyl derivatives of this invention provide enhanced stability to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall

activity *in vivo*. Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

In another aspect, the invention features hammerhead motifs having enzymatic activity having ribonucleotides at locations shown in Figure 80 at 5, 6, 8, 12, and 15.1, and having substituted ribonucleotides at other positions in the core and in the substrate binding arms if desired. (The term "core" refers to positions between bases 3 and 14 in Figure 80, and the binding arms correspond to the bases from the 3'-end to base 15.1, and from the 5'-end to base 2). Applicant has found that use of ribonucleotides at these five locations in the core provide a molecule having sufficient enzymatic activity even when modified nucleotides are present at other sites in the motif. Other such combinations of useful ribonucleotides can be determined as described by Usman *et al. supra*.

Figure 80 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. This is not to be taken as an indication that the Figure is prior art to the pending claims, or that the art discussed is prior art to those claims. Referring to Figure 80 the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 2'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Although substitutions of any nucleotide with any of the modified nucleotides shown in Figure 81 are possible, and were indeed synthesized, the basic structure composed of primarily 2'-O-Me nucleotides with selected substitutions was chosen to maintain maximal catalytic activity (Yang *et al. Biochemistry* 1992, 31, 5005-5009 and Paoletta *et al. , EMBO J.* 1992, 11, 1913-1919) and ease of synthesis, but is not limiting to this invention.

Ribozymes from Figure 80 and Table 45 were synthesized and assayed for catalytic activity and nuclease resistance. With the exception of entries 8 and 17, all of the modified ribozymes retained at least 1/10 of the wild-type catalytic activity. From Table 45, all 2'-modified ribozymes showed very large and significant increases in stability in human serum (shown) and in the other fluids described below (Example 55, data not shown). The order of most aggressive nuclease activity was fetal bovine

serum, > human serum > human plasma > human synovial fluid. As an overall measure of the effect of these 2'-substitutions on stability and activity, a ratio B was calculated (Table 45). This B value indicated that all modified ribozymes tested had significant, >100 - >1700 fold, increases in overall stability and activity. These increases in B indicate that the lifetime of these modified ribozymes *in vivo* are significantly increased which should lead to a more pronounced biological effect.

More general substitutions of the 2'-modified nucleotides from Figure 81 also increased the $t_{1/2}$ of the resulting modified ribozymes. However the catalytic activity of these ribozymes was decreased > 10-fold.

In Figure 86 compound 37 may be used as a general intermediate to prepare derivatized 2'-C-alkyl phosphoramidites, where X is CH₃, or an alkyl, or other group described above.

The following are non-limiting examples showing the synthesis of nucleic acids using 2'-C-alkyl substituted phosphoramidites, the syntheses of the amidites, their testing for enzymatic activity and nuclease resistance.

Example 53: Synthesis of Hammerhead Ribozymes Containing 2'-Deoxy-2'-Alkyl nucleotides & Other 2'-Modified Nucleotides

The method of synthesis used generally follows the procedure for normal RNA synthesis as described in Usman, N.; Ogilvie, K.K.; Jiang, M.-Y.; Cedergren, R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 10, 12, 17, 22, 31, 18, 26, 32, 36 and 38). Other 2'-modified phosphoramidites were prepared according to: 3 & 4, Eckstein *et al.* *International Publication* No. WO 92/07065; and 5 Kois *et al.* *Nucleosides & Nucleotides* 1993, 12, 1093-1109. The average stepwise coupling yields were ~98%. The 2'-substituted phosphoramidites were incorporated into hammerhead ribozymes as shown in Figure 80. However, these 2'-alkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense

oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 54: Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-
5 end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched
on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions
were 1 mM, 200 nM, 40 nM or 8 nM and the final substrate RNA
concentrations were ~ 1 nM. Total reaction volumes were 50 mL. The
assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were
10 initiated by mixing substrate and ribozyme solutions at t = 0. Aliquots of 5
mL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each time
point was quenched in formamide loading buffer and loaded onto a 15%
denaturing polyacrylamide gel for analysis. Quantitative analyses were
performed using a phosphorimager (Molecular Dynamics).

15 Example 55: Stability Assay

500 pmol of gel-purified 5'-end-labeled ribozymes were precipitated
in ethanol and pelleted by centrifugation. Each pellet was resuspended in
20 mL of appropriate fluid (human serum, human plasma, human synovial
fluid or fetal bovine serum) by vortexing for 20 s at room temperature. The
20 samples were placed into a 37 °C incubator and 2 mL aliquots were
withdrawn after incubation for 0, 15, 30, 45, 60, 120, 240 and 480 m.
Aliquots were added to 20 mL of a solution containing 95% formamide and
0.5X TBE (50 mM Tris, 50 mM borate, 1 mM EDTA) to quench further
nuclease activity and the samples were frozen until loading onto gels.
25 Ribozymes were size-fractionated by electrophoresis in 20%
acrylamide/8M urea gels. The amount of intact ribozyme at each time point
was quantified by scanning the bands with a phosphorimager (Molecular
Dynamics) and the half-life of each ribozyme in the fluids was determined
by plotting the percent intact ribozyme vs the time of incubation and
30 extrapolation from the graph.

Example 56: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-O-Phenoxythio-
carbonyl-Uridine (7)

To a stirred solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-
uridine, 6, (15.1 g, 31 mmol, synthesized according to *Nucleic Acid*

Chemistry, ed. Leroy Townsend, 1986 pp. 229-231) and dimethylamino-pyridine (7.57 g, 62 mmol) a solution of phenylchlorothionoformate (5.15 mL, 37.2 mmol) in 50 mL of acetonitrile was added dropwise and the reaction stirred for 8 h. TLC (EtOAc:hexanes / 1:1) showed disappearance of the starting material. The reaction mixture was evaporated, the residue dissolved in chloroform, washed with water and brine, the organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica gel with EtOAc:hexanes / 2:1 as eluent to give 16.44 g (85%) of 7.

10 Example 57: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl -Uridine (8)

To a refluxing, under argon, solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-O-phenoxythiocarbonyl-uridine, 7, (5 g, 8.03 mmol) and allyltributyltin (12.3 mL, 40.15 mmol) in dry toluene, benzoyl peroxide (0.5 g) was added portionwise during 1 h. The resulting mixture was allowed to reflux under argon for an additional 7-8 h. The reaction was then evaporated and the product 8 purified by flash chromatography on silica gel with EtOAc:hexanes / 1:3 as eluent. Yield 2.82 g (68.7%).

Example 58: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine (9)

20 A solution of 8 (1.25 g, 2.45 mmol) in 10 mL of dry tetrahydrofuran (THF) was treated with a 1 M solution of tetrabutylammoniumfluoride in THF (3.7 mL) for 10 m at room temperature. The resulting mixture was evaporated, the residue was loaded onto a silica gel column, washed with 1 L of chloroform, and the desired deprotected compound was eluted with 25 chloroform:methanol / 9:1. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched with methanol (20 mL), evaporated, dissolved in chloroform, washed with 30 5% aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel, EtOAc:hexanes / 1:1 as eluent, to give 0.85 g (57%) of 9 as a white foam.

Example 59: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (10)

5'-O-Dimethoxytrityl-2'-C-allyl-uridine (0.64 g, 1.12 mmol) was dissolved in dry dichloromethane under dry argon. N,N-Diisopropylethylamine (0.39 mL, 2.24 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.35 mL, 1.57 mmol) was added dropwise to the stirred reaction solution and stirring was continued for 2 h at RT. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using a gradient of 10-60% EtOAc in hexanes containing 1% triethylamine mixture as eluent. Yield: 0.78 g (90%), white foam.

Example 60: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl-N⁴-Acetyl-Cytidine (11)

15 Triethylamine (6.35 mL, 45.55 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (5.66 g, 81.99 mmol) and phosphorous oxychloride (0.86 mL, 9.11 mmol) in 50 mL of anhydrous acetonitrile. To the resulting suspension a solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-C-allyl uridine (2.32 g, 4.55 mmol) in 30 mL of acetonitrile was added dropwise and the reaction mixture was stirred for 4 h at room temperature. The reaction was concentrated *in vacuo* to a minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The resulting foam was dissolved in 50 mL of 1,4-dioxane and treated with 29% aq. NH₄OH overnight at room temperature. TLC (chloroform:methanol / 9:1) showed complete conversion of the starting material. The solution was evaporated, dried by coevaporation with anhydrous pyridine and acetylated with acetic anhydride (0.52 mL, 5.46 mmol) in pyridine overnight. The reaction mixture was quenched with methanol, evaporated, the residue was dissolved in chloroform, washed with sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, evaporated to dryness and purified by flash chromatography on silica gel (3% MeOH in chloroform). Yield 2.3 g (90%) as a white foam.

Example 61: 5'-O-Dimethoxytrityl-2'-C-Allyl-N⁴-Acetyl-Cytidine

This compound was obtained analogously to the uridine derivative 9 in 55% yield.

Example 62: 5'-O-Dimethoxytrityl-2'-C-allyl-N⁴-Acetyl-Cytidine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (12)

2'-O-Dimethoxytrityl-2'-C-allyl-N⁴-acetyl cytidine (0.8 g, 1.31 mmol) was dissolved in dry dichloromethane under argon. N,N-Diisopropylethylamine (0.46 mL, 2.62 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.38 mL, 1.7 mmol) was added dropwise to a stirred reaction solution and stirring was continued for 2 h at room temperature. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using chloroform:ethanol / 98:2 with 2% triethylamine mixture as eluent. Yield: 0.91 g (85%), white foam.

Example 63: 2'-Deoxy-2'-Methylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine 14 (Hansske, F.; Madej, D.; Robins, M. J. *Tetrahedron* 1984, 40, 125 and Matsuda, A.; Takenuki, K.; Tanaka, S.; Sasaki, T.; Ueda, T. *J. Med. Chem.* 1991, 34, 812) (2.2 g, 4.55 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-uridine (1.0 g, 3.3 mmol, 72.5%) was eluted with 20% MeOH in CH₂Cl₂.

Example 64: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine (15)

2'-Deoxy-2'-methylene-uridine (0.91 g, 3.79 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes as eluant to yield 15 (0.43 g, 0.79 mmol, 22%).

Example 65: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (17)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl- β -D-ribofuranosyl)-uracil (0.43 g, 0.8 mmol) dissolved in dry CH_2Cl_2 (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.28 mL, 1.6 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.25 mL, 1.12 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.3 g, 0.4 mmol, 50%) was purified by flash column chromatography over silica gel using a 25-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.42 (CH_2Cl_2 : MeOH / 15:1)

Example 66: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine 14 (1.92 g, 12.6 mmol) and triphenylphosphine (2.5 g, 9.25 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol, 70%) eluted with 25% hexanes in EtOAc.

Example 67: 2'-Deoxy-2'-Difluoromethylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol, 68%) was eluted with 20% MeOH in CH_2Cl_2 .

Example 68: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine (16)

2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.42 g, 4.18 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture

was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 40% EtOAc:hexanes as eluant to yield 5'-O-DMT-2'-deoxy-2'-difluoromethylene-uridine 16 (1.05 g, 1.8 mmol, 45%).

Example 69: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (18)

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.577 g, 1 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.36 mL, 2 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.44 mL, 1.4 mmol). The reaction mixture was stirred for 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.404 g, 0.52 mmol, 52%) was purified by flash chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂: MeOH / 15:1).

Example 70: 2'-Deoxy-2'-Methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-4-N-Acetyl-Cytidine 20

Triethylamine (4.8 mL, 34 mmol) was added to a solution of POCl₃ (0.65 mL, 6.8 mmol) and 1,2,4-triazole (2.1 g, 30.6 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) uridine 19 (1.65 g, 3.4 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at room temperature for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved in dioxane (10 mL) and aq. ammonia (20 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (3 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5 mL). The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The

organic extracts were dried over Na_2SO_4 , concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 20 (1.3 g, 2.5 mmol, 73%) was eluted with 20% EtOAc in hexanes.

5 Example 71: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine 21

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 20 (1.3 g, 2.5 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The
10 residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol, 80%) was eluted with 10% MeOH in CH_2Cl_2 . 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol) was dissolved in pyridine (10 mL) and a
15 solution of DMT-Cl (0.81 g, 2.4 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH_2Cl_2 (100 mL) and washed with sat. NaHCO_3 (50 mL), water (50 mL) and brine (50 mL). The
20 organic extracts were dried over MgSO_4 , concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield 21 (0.88 g, 1.5 mmol, 75%).

Example 72: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine 3'-(2-Cyanoethyl-N,N-diisopropylphosphoramidite) (22)

25 1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-N-acetyl-cytosine 21 (0.88 g, 1.5 mmol) dissolved in dry CH_2Cl_2 (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.8 mL, 4.5 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction
30 mixture was stirred 2 h at room temperature and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product 22 (0.82 g, 1.04 mmol, 69%) was purified by flash chromatography over silica gel using 50-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.36 (CH_2Cl_2 :MeOH / 20:1).

Example 73: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyl
disiloxane-1,3-diyl)-4-N-Acetyl-Cytidine (24)

Et₃N (6.9 mL, 50 mmol) was added to a solution of POCl₃ (0.94 mL, 10 mmol) and 1,2,4-triazole (3.1 g, 45 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine 23 ([described in example 14] 2.6 g, 5 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at RT for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved in dioxane (20 mL) and aq. ammonia (30 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (5 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5 mL). The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9 mmol, 78%) was eluted with 20% EtOAc in hexanes.

Example 74: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine (25)

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol, 72%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.03 g, 3.1 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO₄, concentrated *in*

vacuo and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield 25 (1.2 g, 1.9 mmol, 68%).

Example 75: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetylcytosine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (26)

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-N-acetylcytosine 25 (0.6 g, 0.97 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.5 mL, 2.9 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product 26, a white foam (0.52 g, 0.63 mmol, 65%) was purified by flash chromatography over silica gel using 30-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂:MeOH / 20:1).

Example 76: 2'-Keto-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine (28)

Acetic anhydride (4.6 mL) was added to a solution of 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (Brown, J.; Christodolou, C.; Jones, S.; Modak, A.; Reese, C.; Sibanda, S.; Ubasawa A. *J. Chem. Soc. Perkin Trans. I* 1989, 1735) (6.2 g, 9.2 mmol) in DMSO (37 mL) and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated *in vacuo*. The residue was taken up in EtOAc and washed with water. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified on a silica gel column to yield 2'-keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 28 (4.8 g, 7.2 mmol, 78%).

Example 77: 2'-Deoxy-2'-methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)

Under a pressure of argon, sec-butyllithium in hexanes (11.2 mL, 14.6 mmol) was added to a suspension of triphenylmethylphosphonium iodide (7.07 g, 17.5 mmol) in THF (25 mL) cooled at -78 °C. The homogeneous orange solution was allowed to warm to -30 °C and a solution of 2'-keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine

- 28 (4.87 g, 7.3 mmol) in THF (25 mL) was transferred to this mixture under argon pressure. After warming to RT, stirring was continued for 24 h. THF was evaporated and replaced by CH₂Cl₂ (250 mL), water was added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl.
- 5 The organic layer was washed with H₂O (20 mL), 5% aqueous NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give the crude compound, which was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 29 (3.86 g, 5.8 mmol, 79%).
- 10

Example 78: 2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine

- 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (3.86 g, 5.8 mmol) dissolved in THF (30 mL) was treated with 1 M TBAF in THF (15 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (1.8 g, 4.3 mmol, 74%) was eluted with 10% MeOH in CH₂Cl₂.
- 15

- 20 Example 79: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)

- 2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (0.75 g, 1.77 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.66 g, 1.98 mmol) in pyridine (10 mL) was added dropwise over 15 m.
- 25 The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as an eluant to yield 29 (0.81 g, 1.1 mmol, 62%).
- 30

Example 80: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (31)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-6-N-(4-*t*-butylbenzoyl)-adenine 29 dissolved in dry CH₂Cl₂ (15 mL) was placed

in a round bottom flask under Ar. Diisopropylethylamine was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite. The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was
5 evaporated to a syrup *in vacuo* (40 °C). The product was purified by flash chromatography over silica gel using 30-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant (0.7 g, 0.76 mmol, 68%). *R*_f 0.45 (CH₂Cl₂: MeOH / 20:1)

10 Example 81: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine 28 (6.7 g, 10 mmol) and triphenylphosphine (2.9 g, 11 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium
15 chlorodifluoroacetate (2.3 g, 15 mmol) in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-
20 (4-*t*-butylbenzoyl)-adenosine (4.1g, 6.4 mmol, 64%) eluted with 15% hexanes in EtOAc.

Example 82: 2'-Deoxy-2'-Difluoromethylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (4.1 g, 6.4 mmol) dissolved in THF
25 (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol, 77%) was eluted
30 with 20% MeOH in CH₂Cl₂.

Example 83: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine (30)

2'-Deoxy-2'-difluoromethylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in

pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The
5 organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as eluant to yield 30 (2.6 g, 3.41 mmol, 69%).

Example 84: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-N-(4-t-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite)
10 (32)

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofurano-
syl)-6-N-(4-t-butylbenzoyl)-adenine 30 (2.6 g, 3.4 mmol) dissolved in dry
CH₂Cl₂ (25 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the
15 dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (1.06 mL, 4.76 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). 32 (2.3 g, 2.4 mmol, 70%) was purified by flash
column chromatography over silica gel using 20-50% EtOAc gradient in
20 hexanes, containing 1% triethylamine, as eluant. R_f 0.52 (CH₂Cl₂: MeOH / 15:1).

Example 85: 2'-Deoxy-2'-Methoxycarbonylmethylidene-3',5'-O-(Tetraiso-
propyldisiloxane-1,3-diyl)-Uridine (33)

Methyl(triphenylphosphoranylidene)acetate (5.4 g, 16 mmol) was
25 added to a solution of 2'-keto-3',5'-O-(tetraisopropyl disiloxane-1,3-diyl)-
uridine 14 in CH₂Cl₂ under argon. The mixture was left to stir at RT for 30
h. CH₂Cl₂ (100 mL) and water were added (20 mL), and the solution was
neutralized with a cooled solution of 2% HCl. The organic layer was
washed with H₂O (20 mL), 5% aq. NaHCO₃ (20 mL), H₂O to neutrality, and
30 brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo*
to give crude product, that was chromatographed on a silica gel column.
Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-
methoxycarbonylmethylidene-3',5'-O-(tetraisopropyl disiloxane-1,3-diyl)-
uridine 33 (5.8 g, 10.8 mmol, 67.5%).

Example 86: 2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (34)

Et₃N·3 HF (3 mL) was added to a solution of 2'-deoxy-2'-methoxycarbonylmethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine 33 (5 g, 9.3 mmol) dissolved in CH₂Cl₂ (20 mL) and Et₃N (15 mL). The
5 resulting mixture was evaporated *in vacuo* after 1 h and chromatographed on a silica gel column eluting 2'-deoxy-2'-methoxycarbonylmethylidene-uridine 34 (2.4 g, 8 mmol, 86%) with THF:CH₂Cl₂ / 4:1.

Example 87: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (35)

10 2'-Deoxy-2'-methoxycarbonylmethylidene-uridine 34 (1.2 g, 4.02 mmol) was dissolved in pyridine (20 mL). A solution of DMT-Cl (1.5 g, 4.42 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken
15 up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 2-5% MeOH in CH₂Cl₂ as an eluant to yield 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine 35 (2.03 g, 3.46 mmol, 86%).

20 Example 88: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (36)

1-(2'-Deoxy-2'-2'-methoxycarbonylmethylidene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uridine 35 (2.0 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine
25 (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.91 mL, 4.08 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) 36 (1.8 g, 2.3 mmol, 67%)
30 was purified by flash column chromatography over silica gel using a 30-60% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.44 (CH₂Cl₂:MeOH / 9.5:0.5).

Example 89: 2'-Deoxy-2'-Carboxymethylidene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine 37

2'-Deoxy-2'-methoxycarbonylmethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine 33 (5.0 g, 10.8 mmol) was dissolved in MeOH (50 mL) and 1 N NaOH solution (50 mL) was added to the stirred solution at RT. The mixture was stirred for 2 h and MeOH removed *in vacuo*. The pH of the aqueous layer was adjusted to 4.5 with 1N HCl solution, extracted with EtOAc (2 x 100 mL), washed with brine, dried over MgSO₄ and concentrated *in vacuo* to yield the crude acid. 2'-Deoxy-2'-carboxymethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine 37 (4.2 g, 7.8 mmol, 73%) was purified on a silica gel column using a gradient of 10-15% MeOH in CH₂Cl₂.

The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan *et al.* PCT WO 94/02595.

Oligonucleotides with 3' and/or 5' Dihalophosphonate

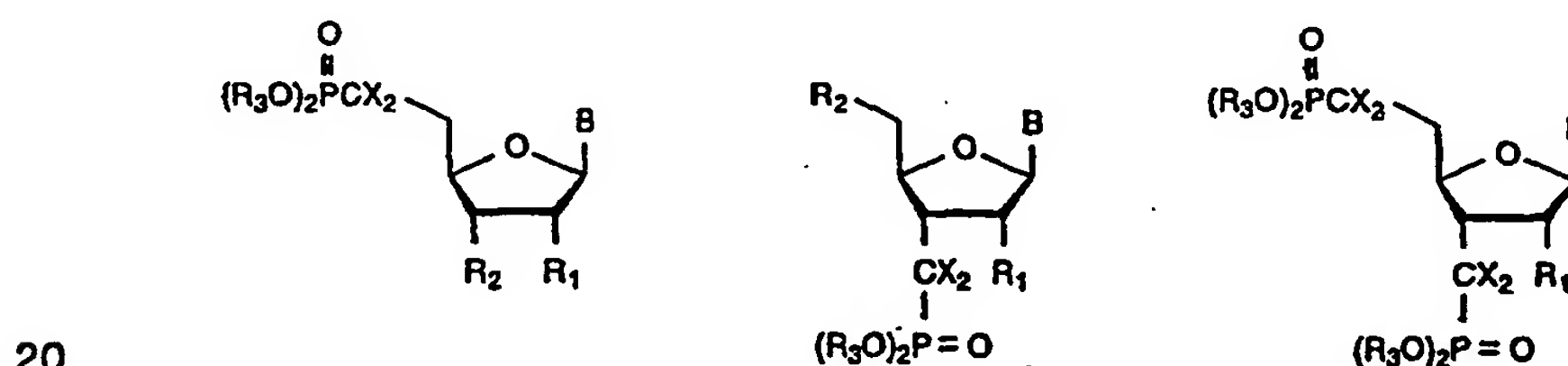
This invention synthesis and uses 3' and/or 5' dihalophosphonate-, e.g., 3' or 5'-CF₂-phosphonate-, substituted nucleotides that maintain or enhance the catalytic activity and/or nuclease resistance of an enzymatic or antisense molecule.

As the term is used in this application, 5'- and/or 3'-dihalophosphonate nucleotide containing ribozymes, deoxyribozymes (see Usman *et al.*, PCT/US94/11649, incorporated by reference herein), and chimeras of nucleotides, are catalytic nucleic molecules that contain 5'- and/or 3'-dihalophosphonate nucleotide components replacing, but not limited to, double-stranded stems, single-stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA or DNA transcript. This invention concerns

nucleic acids formed of standard nucleotides or modified nucleotides, which also contain at least one 5'-dihalophosphonate and/or one 3'-dihalophosphonate group.

The synthesis of 1-O-Ac-2,3-di-O-Bz-D-ribofuranose 5-d-
 5 5-dihalomethylphosphonate in three steps from 1-O-methyl-2,3-O-isopropylidene-β-D-ribofuranose 5-deoxy-5-dihalomethylphosphonate is described (e.g., for the difluoro, in Figure 87). Condensation of this suitably derivatized sugar with silylated pyrimidines and purines affords novel nucleoside 5'-deoxy-5'-dihalomethylphosphonates. These intermediates
 10 may be incorporated into catalytic or antisense nucleic acids by either chemical (conversion of the nucleoside 5'-deoxy-5'-dihalomethylphosphonates into suitably protected phosphoramidites 12a or solid supports 12b, e.g., Figure 88) or enzymatic means (conversion of the nucleoside 5'-deoxy-5'-dihalomethylphosphonates into their
 15 triphosphates, e.g., 14 Figure 89, for T7 transcription).

Thus, in one aspect the invention features 5' and/or 3'-dihalonucleotides and nucleic acids containing such 5' and/or 3'-dihalonucleotides. The general structure of such molecules is shown below.



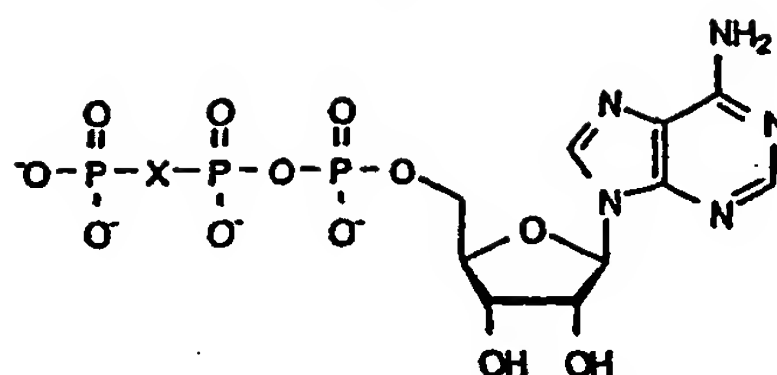
25 where R_1 is H, OH, or R, where R is a hydroxyl protecting group, e.g., acyl, alkylsilyl, or carbonate; each R_2 is separately H, OH, or R; each R_3 is separately a phosphate protecting group, e.g., methyl, ethyl, cyanoethyl, p-nitrophenyl, or chlorophenyl; each X is separately any halogen; and each B is any nucleotide base.

The invention in particular features nucleic acid molecules having such modified nucleotides and enzymatic activity. In a related aspect the invention features a method for synthesis of such nucleoside 5'-deoxy-5'-
 30 dihalo and/or 3'-deoxy-3'-dihalophosphonates by condensing a

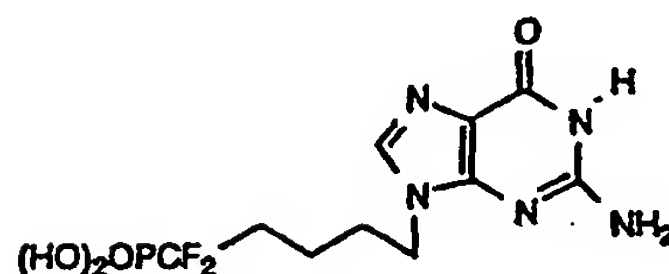
dihalophosphonate-containing sugar with a pyrimidine or a purine under conditions suitable to form a nucleoside 5'-deoxy-5'-dihalophosphonate and/or a 3'-deoxy-3'-dihalophosphonate.

Phosphonic acids may exhibit important biological properties because of their similarity to phosphates (Engel, *Chem. Rev.* 1977, 77, 349-367). Blackburn and Kent (*J. Chem. Soc., Perkin Trans.* 1986, 913-917) indicate that based on electronic and steric considerations α -fluoro and α,α -difluoromethylphosphonates might mimic phosphate esters better than the corresponding phosphonates. Analogues of pyro- and triphosphates 1, where the bridging oxygen atoms are replaced by a difluoromethylene group, have been employed as substrates in enzymatic processes (Blackburn *et al.*, *Nucleosides & Nucleotides* 1985, 4, 165-167; Blackburn *et al.*, *Chem. Scr.* 1986, 26, 21-24). 9-(5,5-Difluoro-5-phosphonopentyl)guanine (2) has been utilized as a multisubstrate analogue inhibitor of purine nucleoside phosphorylase (Halazy *et al.*, *J. Am. Chem. Soc.* 1991, 113, 315-317). Oligonucleotides containing methylene groups in place of phosphodiester 5'-oxygens are resistant toward nucleases that cleave phosphodiester linkages between phosphorus and the 5'-oxygen (Breaker *et al.*, *Biochemistry* 1993, 32, 9125-9128), but can still form stable complexes with complementary sequences. Heinemann *et al.* (*Nucleic Acids Res.* 1991, 19, 427-433) found that a single 3'-methylenephosphonate linkage had a minor influence on the conformation of a DNA octamer double helix.

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1



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3

One common synthetic approach to α,α -difluoro-alkylphosphonates features the displacement of a leaving group from a suitable reactive substrate by diethyl (lithiodifluoromethyl)phosphonate (3) (Obayashi *et al.*, *Tetrahedron Lett.* 1982, 23, 2323-2326). However, our attempts to synthesize nucleoside 5'-deoxy-5'-difluoro-methylphosphonates from 5'-deoxy-5'-iodonucleosides using 3 were unsuccessful, *i.e.* starting compounds were quantitatively recovered. The reaction of nucleoside 5'-aldehydes with 3, according to the procedure of Martin *et al.* (Martin *et al.*, *Tetrahedron Lett.* 1992, 33, 1839-1842), led to a complex mixture of products. Recently, the synthesis of sugar α,α -difluoroalkylphosphonates from primary sugar triflates using 3 was described (Berkowitz *et al.*, *J. Org. Chem.* 1993, 58, 6174-6176). Unfortunately, our experience is that nucleoside 5'-triflates are too unstable to be used in these syntheses.

The following are non-limiting examples showing the synthesis of nucleoside 5'-deoxy-5'-difluoromethyl-phosphonates. Those in the art will recognize that equivalent methods can be readily devised based upon

these examples. These examples demonstrate that it is possible to achieve synthesis of 5'-deoxy-5'-difluoro derivatives in good yield and thus guide those in the art to such equivalent methods. The examples also indicate utility of such synthesis to provide useful oligonucleotides as described above.

Those in the art will recognize that useful modified enzymatic nucleic acids can now be designed, much as described by Draper et al., PCT/US94/13129 hereby incorporated by reference herein (including drawings).

10 Example 90: Synthesis of Nucleoside 5'-Deoxy-5'-difluoromethylphosphonates

Referring to Fig. 87, we synthesized a suitable glycosylating agent from the known D-ribose α,α -difluoromethylphosphonate (4) (Martin et al., *Tetrahedron Lett.* 1992, 33, 1839-1842) which served as a key intermediate for the synthesis of nucleoside 5'-difluoromethylphosphonates.

Methyl 2,3-O-isopropylidene- β -D-ribofuranose α,α -difluoromethylphosphonate (4) was synthesized from the 5-aldehyde according to the procedure of Martin et al. (*Tetrahedron Lett.* 1992, 33, 1839-1842) (Figure 87). Removal of the isopropylidene group was accomplished under mild conditions (I_2 -MeOH, reflux, 18 h (Szarek et al., *Tetrahedron Lett.* 1986, 27, 3827) or Dowex 50 WX8 (H^+), MeOH, RT (about 20-25°C), 3 days) in 72% yield. The anomeric mixture thus obtained was benzoylated with benzoyl chloride/pyridine to afford the 2,3-di-O-benzoyl derivative, which was subjected to mild acetolysis conditions (Walczak et al., *Synthesis*, 1993, 790-792) (Ac_2O , AcOH, H_2SO_4 , EtOAc, 0°C. The desired 1-O-acetyl-2,3-di-O-benzoyl-D-ribofuranose difluoromethylphosphonate (5) was obtained in quantitative yield as an anomeric mixture. These derivatives were used for selective glycosylation of silylated uracil and N^4 -acetylcytosine under Vorbrüggen conditions (Vorbrüggen, *Nucleoside Analogs. Chemistry, Biology and Medical Applications*, NATO ASI Series A, 26, Plenum Press, New York, London, 1980; pp. 35-69. The use of $F_3CSO_2OSi(CH_3)_3$ as a glycosylation catalyst is precluded because it is expected to lead to the undesired 1-ethyluracil or 9-ethyladenine byproducts: Podyukova, et al., *Tetrahedron*

Letf. 1987, 28, 3623-3626 and references cited therein) (SnCl_4 as a catalyst, boiling acetonitrile) to yield β -nucleosides (62% 6a, 75% 6b). Glycosylation of silylated N^6 -benzoyladenine under the same conditions yielded a mixture of N-9 isomer 6c and N-7 isomer 7 in 34% and 15% yield, respectively. The above nucleotides were successfully deprotected using trimethylsilylbromide for the cleavage of the ethyl groups, followed by treatment with ammonia-methanol to remove the acyl protecting groups. Nucleoside 5'-deoxy-5'-difluoromethylphosphonates 8 were finally purified on a DEAE Sephadex A-25 (HCO_3^-) column using a 0.01-0.25 M TEAB gradient for elution and obtained as their sodium salts (82% 8a; 87% 8b; 82% 8c).

Selected analytical data: ^{31}P -NMR (^{31}P) and ^1H -NMR (^1H) were recorded on a Varian Gemini 400. Chemical shifts in ppm refer to H_3PO_4 and TMS, respectively. Solvent was CDCl_3 unless otherwise noted. 5: ^1H δ 8.07-7.28 (m, Bz), 6.66 (d, $J_{1,2}$ 4.5, $\alpha\text{H}1$), 6.42 (s, $\beta\text{H}1$), 5.74 (d, $J_{2,3}$ 4.9, $\beta\text{H}2$), 5.67 (dd, $J_{3,2}$ 4.9, $J_{3,4}$ 6.6, $\beta\text{H}3$), 5.63 (dd, $J_{3,2}$ 6.7, $J_{3,4}$ 3.6, $\alpha\text{H}3$), 5.57 (dd, $J_{2,1}$ 4.5, $J_{2,3}$ 6.7, $\alpha\text{H}2$), 4.91 (m, H4), 4.30 (m, CH_2CH_3), 2.64 (m, CH_2CF_2), 2.18 (s, βAc), 2.12 (s, αAc), 1.39 (m, CH_2CH_3). ^{31}P δ 7.82 (t, $J_{\text{P,F}}$ 105.2), 7.67 (t, $J_{\text{P,F}}$ 106.5). 6a: ^1H δ 9.11 (s, 1H, NH), 8.01 (m, 11H, Bz, H6), 5.94 (d, $J_{1',2'}$ 4.1, 1H, H1'), 5.83 (dd, $J_{5,6}$ 8.1, 1H, H5), 5.79 (dd, $J_{2',1'}$ 4.1, $J_{2',3'}$ 6.5, 1H, H2'), 5.71 (dd, $J_{3',2'}$ 6.5, $J_{3',4'}$ 6.4, 1H, H3'), 4.79 (dd, $J_{4',3'}$ 6.4, $J_{4',\text{F}}$ 11.6, 1H, H4'), 4.31 (m, 4H, CH_2CH_3), 2.75 (tq, $J_{\text{H,F}}$ 19.6, 2H, CH_2CF_2), 1.40 (m, 6H, CH_2CH_3). ^{31}P δ 7.77 (t, $J_{\text{P,F}}$ 104.0). 8c: ^{31}P (vs DSS) (D_2O) δ 5.71 (t, $J_{\text{P,F}}$ 87.9).

Compound 7 was deacylated with methanolic ammonia yielding the product that showed λ_{max} (H_2O) 271 nm and λ_{min} 233 nm, confirming that the site of glycosylation was N-7.

Example 91: Synthesis of Nucleic Acids Containing Modified Nucleotide Containing Cores

The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman *et al.*, *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe *et al.*, *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (Figure 88 and Janda *et al.*, *Science* 1989, 244:437-440.). These

nucleoside 5'-deoxy-5'-difluoromethylphosphonates may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group 1 or Group 2 introns, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

5 Example 92: Synthesis of Modified Triphosphate

The triphosphate derivatives of the above nucleotides can be formed as shown in Fig. 89, according to known procedures. *Nucleic Acid Chem.*, Leroy B. Townsend, John Wiley & Sons, New York 1991, pp. 337-340; *Nucleotide Analogs*, Karl Heinz Scheit; John Wiley & Sons New York 1980, pp. 211-218.

Equivalent synthetic schemes for 3' dihalophosphonates are shown in Figures 90 and 91 using art recognized nomenclature. The conditions can be optimized by standard procedures.

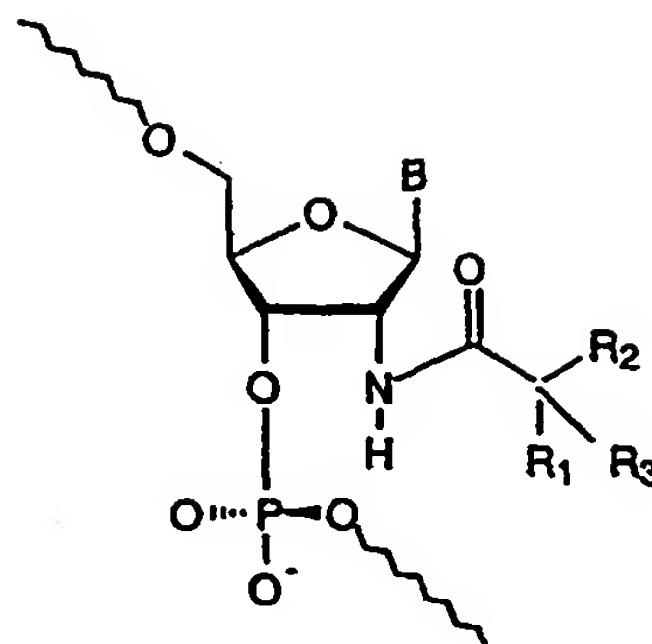
The nucleoside dihalophosphonates described herein are advantageous as modified nucleotides in any nucleic acid structure, e.g., catalytic or antisense, since they are resistant to exo- and endonucleases that normally degrade unmodified nucleic acids *in vivo*. They also do not perturb the normal structure of the nucleic acid in which they are incorporated thereby maintaining any activity associated with that structure.

20 These compounds may also be of use as monomers as antiviral and/or antitumor drugs.

Oligonucleotides with Amido or Peptido Modification

This invention replaces 2'-hydroxyl group of a ribonucleotide moiety with a 2'-amido or 2'-peptido moiety. In other embodiments, the 3' and 5' portions of the sugar of a nucleotide may be substituted, or the phosphate group may be substituted with amido or peptido moieties. Generally, such a nucleotide has the general structure shown in Formula I below:

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FORMULA I

The base (B) is any one of the standard bases or is a modified nucleotide base known to those in the art, or can be a hydrogen group. In addition, either R_1 or R_2 is H or an alkyl, alkene or alkyne group containing between 2 and 10 carbon atoms, or hydrogen, an amine (primary, secondary or tertiary, *e.g.*, R_3NR_4 where each R_3 and R_4 independently is hydrogen or an alkyl, alkene or alkyne having between 2 and 10 carbon atoms, or is a residue of an amino acid, *i.e.*, an amide), an alkyl group, or an amino acid (D or L forms) or peptide containing between 2 and 5 amino acids. The zigzag lines represent hydrogen, or a bond to another base or other chemical moiety known in the art. Preferably, one of R_1 , R_2 and R_3 is an H, and the other is an amino acid or peptide.

Applicant has recognized that RNA can assume a much more complex structural form than DNA because of the presence of the 2'-hydroxyl group in RNA. This group is able to provide additional hydrogen bonding with other hydrogen donors, acceptors and metal ions within the RNA molecule. Applicant now provides molecules which have a modified amine group at the 2' position, such that significantly more complex structures can be formed by the modified oligonucleotide. Such modification with a 2'-amido or peptido group leads to expansion and enrichment of the side-chain hydrogen bonding network. The amide and peptide moieties are responsible for complex structural formation of the oligonucleotide and can form strong complexes with other bases, and interfere with standard base pairing interactions. Such interference will allow the formation of a complex nucleic acid and protein conglomerate.

Oligonucleotides of this invention are significantly more stable than existing oligonucleotides and can potentially form biologically active bioconjugates not previously possible for oligonucleotides. They may also be used for *in vitro* selection of unique aptamers, that is, randomly generated oligonucleotides which can be folded into an effective ligand for a target protein, nucleic acid or polysaccharide.

Thus, in one aspect, the invention features an oligonucleotide containing the modified base shown in Formula 1, above.

In other aspects, the oligonucleotide may include a 3' or 5' nucleotide having a 3' or 5' located amino acid or aminoacyl group. In all these aspects, as well as the 2'-modified nucleotide, it will be evident that various standard modifications can be made. For example, an "O" may be replaced with an S, the sugar may lack a base (i.e., abasic) and the phosphate moiety may be modified to include other substitutions (see Sproat, *supra*).

Example 93: General procedure for the preparation of 2'-aminoacyl-2'-deoxy-2'-aminonucleoside conjugates.

Referring to Fig. 92, to the solution of 2'-deoxy-2'-amino nucleoside (1 mmol) and N-Fmoc L- (or D-) amino acid (1 mmol) in methanol [dimethylformamide (DMF) and tetrahydrofuran (THF) can also be used], 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) [or 1-isobutyloxycarbonyl-2-isobutyloxy-1,2-dihydroquinoline (IIDQ)] (2 mmol) is added and the reaction mixture is stirred at room temperature or up to 50 °C from 3-48 hours. Solvents are removed under reduced pressure and the residual syrup is chromatographed on the column of silica-gel using 1-10 % methanol in dichloromethane. Fractions containing the product are concentrated yielding a white foam with yields ranging from 85 to 95 %. Structures are confirmed by ¹H NMR spectra of conjugates which show correct chemical shifts for nucleoside and aminoacyl part of the molecule. Further proofs of the structures are obtained by cleaving the aminoacyl protecting groups under appropriate conditions and assigning ¹H NMR resonances for the fully deprotected conjugate.

Partially protected conjugates described above are converted into their 5'-O-dimethoxytrityl derivatives and into 3'-phosphoramidites using standard procedures (Oligonucleotide Synthesis: A Practical Approach,

M.J. Gait ed.; IRL Press, Oxford, 1984). Incorporation of these phosphoramidites into RNA was performed using standard protocols (Usman *et al.*, 1987 *supra*).

5 A general deprotection protocol for oligonucleotides of the present invention is described in Fig. 93.

The scheme shows synthesis of conjugate of 2'-d-2'-aminouridine. This is meant to be a non-limiting example, and those skilled in the art will recognize that, variations to the synthesis protocol can be readily generated to synthesize other nucleotides (e.g., adenosine, cytidine, 10 guanosine) and/or abasic moieties.

Example 94: RNA cleavage by hammerhead ribozymes containing 2'-aminoacyl modifications.

Hammerhead ribozymes targeted to site N (see Fig. 94) are synthesized using solid-phase synthesis, as described above. U4 and U7 15 positions are modified, individually or in combination, with either 2'-NH-alanine or 2'-NH-lysine.

RNA cleavage assay *in vitro*: Substrate RNA is 5' end-labeled using [γ -³²P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace 20 amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂. The reaction is initiated by mixing the 25 ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of 30 time.

Referring to Fig. 95, hammerhead ribozymes containing 2'-NH-alanine or 2'-NH-lysine modifications at U4 and U7 positions cleave the target RNA efficiently.

Sequences listed in Figure 94 and the modifications described in Figure 95 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing
5 other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 95: Aminoacylation of 3'-ends of RNA

I. Referring to Fig. 96, 3'-OH group of the nucleotide is converted to succinate as described by Gait, *supra*. This can be linked with amino-alkyl
10 solid support (for example: CpG). Zig-zag line indicates linkage of 3'OH group with the solid support.

II. Preparation of aminoacyl-derivatized solid support

A) Synthesis of O-Dimethoxytrityl (O-DMT) amino acids

15 Referring to Fig. 97, to a solution of L- (or D-) serine, tyrosine or threonine (2 mmol) in dry pyridine (15 ml) 4,4'-dimethoxytrityl chloride (3 mmol) is added and the reaction mixture is stirred at RT (about 20-25°C) for 16 h. Methanol (10 ml) is then added and the solution evaporated under reduced pressure. The residual syrup was partitioned between 5% aq.
20 NaHCO₃ and dichloromethane, organic layer was washed with brine, dried (Na₂SO₄) and concentrated *in vacuo*. The residue is purified by flash silicagel column chromatography using 2-10% methanol in dichloromethane (containing 0.5 % pyridine). Fractions containing product are combined and concentrated *in vacuo* to yield white foam (75-85 %
25 yield).

B) Preparation of the solid support and its derivatization with amino acids

Referring to Fig. 97, the modified solid support (has an OH group instead of the standard NH₂ end group) was prepared according to Haralambidis et al., *Tetrahedron Lett.* 1987, 28, 5199, (P denotes
30 aminopropyl CPG or polystyrene type support). O-DMT or NH-monomethoxytrityl (NH-MMT amino acid was attached to the above solid support using standard procedures for derivatization of the solid support (Gait, 1984, *supra*) creating a base-labile ester bond between amino acids

and the support. This support is suitable for the construction of RNA/DNA chain using suitably protected nucleoside phosphoramidites.

Example 96: Aminoacylation of 5'-ends of RNA

I. Referring to Fig. 98, 5'-amino-containing sugar moiety was synthesized as described (Mag and Engels, 1989 *Nucleic Acids Res.* 17, 5973). Aminoacylation of the 5'-end of the monomer was achieved as described above and RNA phosphoramidite of the 5'-aminoacylated monomer was prepared as described by Usman *et al.*, 1987 *supra*. The phosphoramidite was then incorporated at the 5'-end of the oligonucleotide using standard solid-phase synthesis protocols described above.

II. Referring to Fig. 99, aminoacyl group(s) is attached to the phosphate group at the 5'-end of the RNA using standard procedures described above.

VII. Reversing Genetic Mutations

Modification of existing nucleic acid sequences can be achieved by homologous recombination. In this process a transfected sequence recombines with homologous chromosomal sequences and can replace the endogenous cellular sequence. Boggs, 8 *International J. Cell Cloning* 80, 1990, describes targeted gene modification. It reviews the use of homologous DNA recombination to correct genetic defects. Banga and Boyd, 89 *Proc. Natl. Acad. Sci. U.S.A.* 1735, 1992, describe a specific example of *in vivo* site-directed mutagenesis using a 50 base oligonucleotide. In this methodology a gene or gene segment is essentially replaced by the oligonucleotide used.

This invention uses a complementary oligonucleotide to position a nucleotide base changing activity at a particular site on a gene (RNA or genomic DNA), such that the nucleotide modifying activity will change (or revert) a mutation to wild-type, or its equivalent. By reversion or change of a mutation, we refer to reversion in a broad sense, such as when a mutation at a second site which leads to functional reversion to a wild type phenotype. Also, due to the degeneracy of the genetic code, a revertant may be achieved by changing any one of the three codon positions. Additionally, creation of a stop codon in a deleterious gene (or transcript) is defined here as reverting a mutant phenotype to wild-type. An example of

this type of reversion is creating a stop codon in a critical HIV proviral gene in a human.

Referring to Figures 100 and 101, broadly there are two approaches to causing a site directed change in order to revert a mutation to wild-type.

5 In one (Fig. 100) the oligonucleotide is used to target RNA specifically. RNA is provided with a complementary (Watson-crick) oligonucleotide sequence to that in the target molecule. In this case the sequence modifying oligonucleotide would (analogously to an antisense oligonucleotide or ribozyme) have to be continuously present to revert the

10 RNA as it is made by the cell. Such a reversion would be transient and would potentially require continuous addition of more sequence modifying oligonucleotide. The transient nature of this approach is an advantage, in that treatment could be stopped by simply removing the sequence modifying oligonucleotide (as with a traditional drug).

15 A second approach targets DNA (Fig. 101) and has the advantage that changes may be permanently encoded in the target cell's genetic code. Thus, a single course (or several courses) of treatment may lead to permanent reversion of the genetic disease. If inadvertent chromosomal mutations are introduced this may cause cancer, mutate other genes, or

20 cause genetic changes in the germ-line (in patients of reproductive age). However, if the base changing activity is a specific methylation that may modulate gene expression it would not necessarily lead to germ-line transmission. See Lewin, Genes, 1983 John Wiley & Sons, Inc. NY pp 493-496.

25 Complementary base pairing to single-stranded DNA or RNA is one method of directing an oligonucleotide to a particular site of DNA. This could occur by a strand displacement mechanism or by targeting DNA when it is single-stranded (such as during replication, or transcription). Another method is using triple-strand binding (triplex formation) to double-

30 stranded DNA, which is an established technique for binding polypyrimidine tracts, and can be extended to recognize all 4 nucleotides. See Povsic, T., Strobel, S., & Dervan, P. (1992). Sequence-specific double-strand alkylation and cleavage of DNA mediated by triple-helix formation. J. Am. Chem. Soc. 114, 5934-5944 (1992). Knorre, D.G., Valentin, V.V.,

35 Valentina, F.Z., Lebedev, A.V. & Federova, O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk,

1993) describe conjugation of reactive groups or enzyme to oligonucleotides and can be used in the methods described herein.

Recently, antisense oligonucleotides have been used to redirect an incorrect splice into order to obtain correct splicing of a splice mutant globin gene *in vitro*. Dominski Z; Kole R (1993) Restoration of correct splicing in thalassemia pre-mRNA by antisense oligonucleotides. Proc Natl Acad Sci U S A 90:8673-7. Analogously, in one preferred embodiment of this invention a complementary oligomer is used to correct an existing mutant RNA, instead of the traditional approach of inhibiting that RNA by antisense.

In either the RNA or DNA mode, after binding to a particular site on the RNA or DNA the oligonucleotide will modify the nucleic acid sequence. This can be accomplished by activating an endogenous enzyme (see Figure 102), by appropriate positioning of an enzyme (or ribozyme) conjugated (or activated by the duplex) to the oligonucleotide, or by appropriate positioning of a chemical mutagen. Specific mutagens, such as nitrous acid which deaminates C to U, are most useful, but others can also be used if inactivation of a harmful RNA is desired.

RNA editing is an naturally occurring event in mammalian cells in which a sequence modifying activity edits a RNA to its proper sequence post-transcriptionally. Higuchi, M., Single, F., Kohler, M., Sommer, B., and Seeburg, P. (1993) RNA Editing of AMPA Receptor Subunit GluR-B: A base-paired intron-exon structure determines position and efficiency Cell 75:1361-1370. The machinery involved in RNA editing can be co-opted by a suitable oligonucleotide in order to promote chemical modification.

The changes in the base created by the methods of this invention cause a change in the nucleotide sequence, either directly, or after DNA repair by normal cellular mechanisms. These changes functionally correct a genetic defect or introduce a stop codon. Thus, the invention is distinct from techniques in which an active chemical group (*e.g.*, an alkylator) is attached to an antisense or triple strand oligonucleotide in order to chemically inactivate the target RNA or DNA.

Thus, this invention creates an alteration to an existing base in a nucleic acid molecule so that the base is read *in vivo* as a different base.

This includes correcting a sequence instead of inactivating a gene but can also include inactivating a deleterious gene.

Thus, in one aspect, the invention features a method for altering in vivo the nucleotide base sequence of a naturally occurring mutant nucleic acid molecule. The method includes contacting the nucleic acid molecule in vivo with an oligonucleotide or peptide nucleic acid or other sequence specific binding molecules able to form a duplex or triplex molecule with the nucleic acid molecule. After formation of the duplex or triplex molecule a base modifying activity chemically or enzymatically alters the targeted base directly, or after nucleic acid repair *in vivo*. This results in the functional alteration of the nucleic acid sequence.

By "alter", as it is used in this context, is meant that one or more chemical moieties in a targeted base, or bases, is altered so that the mutant nucleic acid will be functionally different. Thus, this is distinct from prior methods of correcting defects in DNA, such as homologous recombination, in which an entire segment of the targeted sequence is replaced with a segment of DNA from the transfected nucleic acid. This is also distinct from other methods that use reactive groups to inactivate a RNA or DNA target, in that this method functionally corrects the sequence of the target, instead of merely damaging it, by causing it to be read by a polymerase as a different base from the original base. As noted above, the naturally occurring enzymes in a cell can be utilized to cause the chemical alteration, examples of which are provided below.

By "functionally alter" is meant that the ability of the target nucleic acid to perform its normal function (*i.e.*, transcription or translation control) is changed. For example, an RNA molecule may be altered so that it can cause production of a desired protein, or a DNA molecule can be altered so that upon DNA repair, the DNA sequence is changed.

By "mutant" it is meant a nucleic acid molecule which is altered in some way compared to equivalent molecules present in a normal individual. Such mutants may be well known in the art, and include, molecules present in individuals with known genetic deficiencies, such as muscular dystrophy, or diabetes and the like. It also includes individuals with diseases or conditions characterized by abnormal expression of a gene, such as cancer, thalassemia's and sickle cell anemia, and cystic

fibrosis. It allows modulation of lipid metabolism to reduce artery disease, treatment of integrated AIDS genomes, and AIDS RNA, and Alzheimer's disease. Thus, this invention concerns alteration of a base in a mutant to provide a "wild type" phenotype and/or genotype. For deleterious conditions this involves altering a base to allow expression or prevent expression as is necessary. When treating an infection, such as HIV, it concerns inactivation of a gene in the HIV RNA by mutation of the mutant (*i.e.*, non-human gene) to a wild type (*i.e.*, no production of a non-human protein). Such modification is performed *in trans* rather than *in cis* as in prior methods.

In preferred embodiments, the oligonucleotide is of a length (at least 12 bases, preferably 17 - 22) sufficient to activate dsRNA deaminase *in vivo* to cause conversion of an adenine base to inosine; the oligonucleotide is an enzymatic nucleic acid molecule that is active to chemically modify a base (see below); the nucleic acid molecule is DNA or RNA; the oligonucleotide includes a chemical mutagen, *e.g.*, the mutagen is nitrous acid; and the oligonucleotide causes deamination of 5-methylcytosine to thymidine, cytosine to uracil, or adenine to inosine, or methylation of cytosine to 5-methylcytosine.

In a most preferred embodiment, the invention features correction of a mutation, rather than inactivation of a target by causing a mutation.

Using *in vitro* directed evolution, it is possible to screen for ribozymes with catalytic activities different than RNA cleavage. Bartel, D. and Szostak, J. (1993) Isolation of new ribozymes from a large pool of random sequences. Science 261:1411-1418. Using these methods of *in vitro* directed evolution, an enzymatic nucleic acid molecule, or ribozyme that mutates bases, instead of cleaving the phosphodiester backbone can be selected. This is a convenient method of obtaining an enzyme with the appropriate base sequence modifying activities for use in the present invention.

Sequence modifying activities can change one nucleotide to another (or modify a nucleotide so that it will be repaired by the cellular machinery to another nucleotide). Sequence modifying activities could also delete or add one or more nucleotides to a sequence. A specific embodiment of adding sequences is described by Sullenger and Cech, PCT/US94/12976

hereby incorporated by reference herein), in which entire exons with wild-type sequence are spliced into a mutant transcript. The present invention features only the addition of a few bases (1 - 3).

Thus, in another aspect, the invention features ribozymes or enzymatic nucleic acid molecules active to change the chemical structure of an existing base in a separate nucleic acid molecule. Applicant is the first to determine that such molecules would be useful, and to provide a description of how such molecules might be isolated.

Molecules used to achieve *in situ* reversion can be delivered using the existing means employed for delivering antisense molecules and ribozymes, including liposomes and cationic lipid complexes. If the *in situ* reverting molecule is composed only of RNA, then expression vectors can be used in a gene therapy protocol to produce the reverting molecules endogenously, analogously to antisense or ribozymes expression vectors. There are several advantages of using such an expression vector, rather than simply replacing the gene through standard gene therapy. Firstly, this approach would limit the production of the corrected gene to cells that already express that gene. Furthermore, the corrected gene would be properly regulated by its natural transcriptional promoter. Lastly, reversion can be used when the mutant RNA creates a dominant gain of function protein (e.g., in sickle cell anemia), where correction of the mutant RNA is necessary to stop the production of the deleterious mutant protein, and allow production of the corrected protein.

Endogenous Mammalian RNA Editing System

It was observed in the mid-1980s that the sequence of certain cellular RNAs were different from the DNA sequence that encodes them. By a process called RNA editing, cellular RNA are post-transcriptionally modified to a) create a translation initiation and termination codons, b) enable tRNA and rRNA to fold into a functional conformation (for a review see Bass, B. L. (1993) In The RNA World, R. Gesteland, R. and Atkins, J. eds. (Cold Spring Harbor, New York; CSH Lab. Press) pp. 383-418). The process of RNA editing includes base modification, deletion and insertion of nucleotides.

Although, the RNA editing process is widespread among lower eukaryotes, very few RNAs (four) have been reported to undergo editing in

mammals (Bass, *supra*). The predominant mode of RNA editing in mammalian system is base modification ($C \rightarrow U$ and $A \rightarrow G$). The mechanism of RNA editing in the mammalian system is postulated to be that $C \rightarrow U$ conversion is catalyzed by cytidine deaminase. The mechanism of conversion of $A \rightarrow G$ has recently been reported for glutamate receptor B subunit (gluR-B) in rat PC12 cells (Higuchi, M. et al. (1993) *Cell* 75, 1361-1370). According to Higuchi gluR-B mRNA precursor attains a structure such that intron 11 and exon 11 can form a stable stem-loop structure. This stem-loop structure is a substrate for a nuclear double strand-specific adenosine deaminase enzyme. The deamination will result in the conversion of $A \rightarrow I$. Reverse transcription followed by double strand synthesis will result in the incorporation of G in place of A.

In the present invention, the endogenous deaminase activity or other such activities can be utilized to achieve targeted base modification.

The following are examples of the invention to illustrate different methods by which *in vivo* conversion of a base can be achieved. These are provided only to clarify specific embodiments of the invention and are not limiting to the invention. Those in the art will recognize that equivalent methods can be readily devised within the scope of the claims.

Example 97: Exploiting cellular dsRNA dependent Adenine to Inosine converter;

An endogenous activity in most mammalian cells and *Xenopus* oocytes converts about 50% of adenines to inosines in double stranded RNA. (Bass, B. L., & Weintraub, H. (1988). An unwinding activity that covalently modifies its double-stranded RNA substrate. *Cell*, 55, 1089-1098.). This activity can be used to cause an *in situ* reversion of a mutation at the RNA level. Referring to Figures 102 and 104, for demonstration purposes a stop codon is incorporated into the coding region of dystrophin, which is fused to the reporter gene luciferase. This stop codon can be reverted by targeting an antisense RNA which is long enough to activate the dsRNA deaminase, which converts Adenines to Inosines. The A to I transition will be read by the ribosome as an A to G transition in some cases and will thereby functionally revert the stop codon. While other A's in this region may be converted to I's and read as G, converting an A to I (G) cannot create a stop codon. The A to I transitions

in the region surrounding the target mutation will create some point mutations, however, the function of the dystrophin protein is rarely inactivated by point mutations.

5 The reverted mRNA was then translated in a cell lysate and assayed for luciferase activity. As evidenced by the dramatic increase in luciferase counts in the graph in figure 103, the A to I transition was read by the ribosome as an A to G transition and the stop codon has successfully been reverted with the lysate treated complex. As a control, an irrelevant non-complementary RNA oligonucleotide was added to the
10 dystrophin/luciferase mRNA. As expected, in this case no translation (luciferase activity) is observed because of the stop codon. As an additional control, the hybrid was not treated with extract, and again no translation (luciferase activity) is observed (Figure 103).

15 While other A's in the targeted region may have been converted to I's and read as G, converting an A to I (G) cannot create a stop codon, so the ribosome will still read through the region. Dystrophin is not generally sensitive to point mutations if the open reading frame is maintained, so a dystrophin protein made from an mRNA reverted by this method should retain full activity.

20 The following detail specifics of the methodology: RNA oligonucleotides were synthesized on a 394 (ABI) synthesizer using phosphoramidite chemistry. The sequence of the synthetic complementary RNA that binds to the mutant dystrophin sequence is as follows (5' to 3'):

25 CCCGCGGTAGATCTTTCTGGAGGCTTACAGTTTTCTACAAACCTCC
CTTCAA (Seq. ID No. 1)

Referring to Figure 104, fifty-nine base pairs of a human dystrophin mutant sequence containing a stop codon was fused in frame to the luciferase coding region using standard cloning technology, into the *Hind* III and *Not* I sites of pRC-CMV (Invitrogen, San Diego, CA). The AUG of
30 luciferase was deleted. The sequences of the insert from the *Hind* III site to the start of the luciferase coding region is (5' to 3'):

GCCCCTGAGGAGCGATGGAGGCCTTGAAGGGAGGTTTGTGGAAAA
CTGTAAGCCTCCAGAAAGATCTACCGCGG (Seq ID No. 2)

This corresponds to base pairs 3649-3708 of normal dystrophin (Entrez ID # 311627) with a *Sac* II site at the 3' end. This plasmid was used as a template for *in vitro* transcription of mRNA using T7 polymerase with the manufacturers protocol (Promega, Madison, WI).

- 5 *Xenopus* nuclear extracts were prepared in 0.5X TGKED buffer (0.5X= 25mM Tris (pH 7.9), 12.5% glycerol, 25 mM KCl, 0.25mM DTT and 0.05mM EDTA), by vortexing nuclei and resuspended in a volume of 0.5X TGKED equal to total cytoplasm volume of the oocytes. Bass, B.L. & Weintraub, H. *Cell* 55, 1089-1098 (1988).
- 10 The target mRNA at 500ng/ul was pre-annealed to 1 micromolar complementary or irrelevant RNA oligonucleotide by heating to 70°C, and allowing it to slowly cool to 37°C over 30 minutes. Fifty nanograms of mRNA pre-annealed to the RNA oligonucleotides was added to 7ul of nuclear extracts containing 1mM ATP, 15mM EDTA, 1600un/ml RNasin
- 15 and 12.5mM Tris pH 8 to a total volume of 12ul. Bass, B.L. & Weintraub, H. *supra*. This mixture, which contains the dsRNA deaminase activity, was incubated for 30 minutes at 25°C. Next, 1.5ul of this mixture was added to a rabbit reticulocyte lysate *in vitro* translation mixture and translated for two hours according to the manufacturers protocol (Life Technologies,
- 20 Gaithersburg, MD), except that an additional 1.3 mM magnesium acetate was added to compensate for the EDTA carried through from the nuclear extract mixture. Luciferase assays were performed on 15ul of extract with the Promega luciferase assay system (Promega, Madison, WI), and luminescence was detected with a 96 well luminometer, and the results are
- 25 displayed in the graph in figure 102.

Example 98: Base changing activities

- The chemical synthesis of antisense and triple-strand forming oligomers conjugated to reactive groups is well studied and characterized (Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova,
- 30 O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk, 1993) and Povsic, T., Strobel, S. & Dervan, P. Sequence-specific double-strand alkylation and cleavage of DNA mediated by triple-helix formation *J. Am. Chem. Soc.* 114, 5934-5944 (1992). Reactive groups such as alkylators that can modify nucleotide
- 35 bases in targeted RNA or DNA have been conjugated to oligonucleotides.

Additionally enzymes that modify nucleic acids have been conjugated to oligonucleotides. (Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova, O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk, 1993). In the past these
5 conjugated chemical groups or enzymes have been used to inactivate DNA or RNA that is specifically targeted by antisense or triple-strand interactions. Below is a list of useful base changing activities that could be used to change the sequence of DNA or RNA targeted by antisense or triple strand interactions, in order to achieve *in situ* reversion of mutations,
10 as described herein (see figure 100-104).

1. Deamination of 5-methylcytosine to create thymidine (performed by the enzyme cytidine deaminase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993). Also, nitrous acid or related compounds promote oxidative deamination of
15 C to be read as T (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.). Additionally hydroxylamine or related compounds can transform C to be read as T (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.)

20 2. Deamination of cytosine to create uracil (performed by the enzyme cytidine deaminase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993) or by chemical groups similar to nitrous acid that promote oxidative deamination (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc.,
25 Boston, 1987, PP.226-230.)

3. Deamination of Adenine to be read like G (Inosine) (as done by the adenosine deaminase, AMP deaminase or the dsRNA deaminating activity (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993).

30 4. Methylation of cytosine to 5-methylcytosine

5. Transforming thymidine (or uracil) to O²-methyl thymidine (or O²-methyl uracil), to be read as cytosine by alkynitrosoureas (Xu, and Swann, Tetrahedron Letters 35:303-306 (1994)).

6. Transforming guanine to 6-O-methyl (or other alkyls) to be read as adenine (Mehta and Ludlum, *Biochimica et Biophysica Acta*, 521:770-778 (1978) which can be done with the mutagen ethyl methane sulfonate (EMS) Microbial Genetics, David Freifelder, Jones and Bartlett
 5 Publishers, Inc., Boston, 1987, PP.226-230.

7. Amination of uracil to cytosine (as performed by the cellular enzyme CTP synthetase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993).

The following are examples of useful chemical modifications that can
 10 be utilized in the present invention. There are a few preferred straightforward chemical modifications that can change one base to another base. Appropriate mutagenic chemicals are placed on the targetting oligonucleotide, e.g., nitrous acid, or a suitable protein with such activity. Such chemicals and proteins can be attached by standard
 15 procedures. These include molecules which introduce fundamental chemical changes, that would be useful independent of the particular technical approach. See Lewin, *Genes*, 1983 John Wiley & Sons, Inc. NY pp 42-48.

The following matrix shows that the chemical modifications noted can
 20 cause transversion reversions (pyrimidine to pyrimidine, or purine to purine) in RNA or DNA. The transversions (pyrimidine to purine, or purine to pyrimidine) are not preferred because these are more difficult chemical transformations. The footnotes refer to the specific desired chemical transformations. The bold footnotes refer to the reaction on the opposite
 25 DNA strand. For example, if one desires to change an A to a G, this can be accomplished at the DNA level by using reaction #5 to change a T to a C in the opposing strand. In this example an A/T base pair goes to A/C, then when the DNA is replicated, or mismatch repair occurs this can become G/C, thus the original A has been converted to a G.

30

ISR matrix

Reverted Base

Mutant base	A	T(U)	C	G
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161

A	-	Transversion	Transversion	DNA ⁵ /RNA ³
T(U)	Transversion	-	DNA ⁵ /RNA ⁷	Transversion
C	Transversion	RNA ² /DNA ⁶	-	Transversion
G	DNA ⁶ /RNA ⁶	Transversion	Transversion	-

- 1 Deamination of 5-methylcytosine to create thymidine.
- 2 Deamination of cytosine to create uracil.
- 3 Deamination of Adenine to be read like G (Inosine).
- 5 4 Methylation of cytosine to 5-methylcytosine.
- 5 5 Transforming thymidine (or uracil) to O²-methyl thymidine (or O²-methyl uracil), to be read as cytosine (Xu, and Swann, Tetrahedron Letters 35:303-306 (1994)).
- 10 6 Transforming guanine to 6-O-methyl (or other alkyls) to be read as adenine (Mehta and Ludlum, Biochimica et Biophysica Acta, 521:770-778 (1978)).
7. 7. Amination of uracil to cytosine. Bass *supra*. fig. 6c.

In Vitro Selection Strategy

- Referring to Figure 105, there is provided a schematic describing an approach to selecting for a ribozyme with such base changing activity. An RNA is designed that folds back on itself (this is similar to approaches already used to select for RNA ligases, Bartel, D. and Szostak, J. (1993) Isolation of new ribozymes from a large pool of random sequences. Science 261:1411-1418). A degenerate loop opposing the base to be modified provides for diversity. After incubating this library of molecules in a buffer, the RNA is reverse transcribed into DNA (that is, using standard *in vitro* evolution protocol. Tuerk and Gold, 249 Science 505, 1990) , and then the DNA is selected for having a base change. A restriction enzyme cleavage and size selection or its equivalent is used to isolate the fraction of DNAs with the appropriate base change. The cycle could then be repeated many times.

The *in vitro* selection (evolution) strategy is similar to approaches developed by Joyce (Beaudry, A. A. and Joyce, G.F. (1992) Science 257, 635-641; Joyce, G. F. (1992) Scientific American 267, 90-97) and Szostak (Bartel, D. and Szostak, J. (1993) Science 261:1411-1418; Szostak, J. W. (1993) TIBS 17, 89-93). Briefly, a random pool of nucleic acids is synthesized wherein, each member contains two domains: a) one domain consists of a region with defined (known) nucleotide sequence; b) the second domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to bind to its target (the region flanking the mutant nucleotide), 2) complementary DNA (cDNA) synthesis and PCR amplification of molecules selected for their base modifying activity, 3) introduction of restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides represented at every position within the random region) or the degeneracy can be partial (Beaudry, A. A. and Joyce, G.F. (1992) Science 257, 635-641). In this invention, the degenerate domain is flanked by regions containing known sequences (see Figure 105), such that the degenerate domain is placed across from the mutant base (the base that is targeted for modification). This random library of nucleic acids is incubated under conditions that ensure folding of the nucleic acids into conformations that facilitate the catalysis of base modification (the reaction protocol may also include certain cofactors like ATP or GTP or an S-adenosyl-methionine (if methylation is desired) in order to make the selection more stringent). Following incubation, nucleic acids are converted into complementary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with base modification (at the mutant base position) can be separated from rest of the population of nucleic acids by using a variety of methods. For example, a restriction endonuclease cleavage site can either be created or abolished as a result of base modification. If a restriction endonuclease site is created as a result of base modification, then the library can be digested with the restriction endonuclease (RE). The fraction of the population that is cleaved by the RE is the population that has been able to catalyze the base modification reaction (active pool). A new piece of DNA (containing oligonucleotide primer binding sites for PCR and RE sites for cloning) is ligated to the termini of the active pool to facilitate PCR amplification and subsequent cycles (if necessary) of selection. The final pool of nucleic acids with the best base modifying activity is cloned in to a plasmid vector

and transformed into bacterial hosts. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

Base modifying enzymatic nucleic acids (identified via *in vitro* selection) can be used to cause the chemical modification *in vivo*.

In addition, the ribozyme could be evolved to specifically bind a protein having an enzymatic base changing activity.

Such ribozymes can be used to cause the above chemical modifications *in vivo*. The ribozymes or above noted antisense-type molecules can be administered by methods discussed in the above referenced art.

VIII. Administration of Nucleic Acids

Applicant has determined that double-stranded nucleic acid lacking a transcription termination signal can be used for continuous expression of the encoded RNA. This is achieved by use of an R-loop, *i.e.*, an RNA molecule non-covalently associated with the double-stranded nucleic acid and which causes localized denaturation ("bubble" formation) within the double stranded nucleic acid (Thomas et al., 1976 Proc. Natl. Acad. Sci. USA 73, 2294). In addition, applicant has determined that the RNA portion of the R-loop can be used to target the whole R-loop complex to a desirable intracellular or cellular site, and aid in cellular uptake of the complex. Further, applicant indicates that expression of enzymatically active RNA or ribozymes can be significantly enhanced by use of such R-loop complexes.

Thus, in one aspect, the invention features a method for introduction of enzymatic nucleic acid into a cell or tissue. A complex of a first nucleic acid encoding the enzymatic nucleic acid and a second nucleic acid molecule is provided. The second nucleic acid molecule has sufficient complementarity with the first nucleic acid to be able to form an R-loop base pair structure under physiological conditions. The R-loop is formed in a region of the first nucleic acid molecule which promotes expression of RNA from the first nucleic acid under physiological conditions. The method further includes contacting the complex with a cell or tissue under

conditions in which the enzymatic nucleic acid is produced within the cell or tissue.

By "complex" is simply meant that the two nucleic acid molecules interact by intermolecular bond formation (such as by hydrogen bonding) between two complementary base-paired sequences. The complex will generally be stable under physiological condition such that it is able to cause initiation of transcription from the first nucleic acid molecule.

The first and second nucleic acid molecules may be formed from any desired nucleotide bases, either those naturally occurring (such as adenine, guanine, thymine and cytosine), or other bases well known in the art, or may have modifications at the sugar or phosphate moieties to allow greater stability or greater complex formation to be achieved. In addition, such molecules may contain non-nucleotides in place of nucleotides. Such modifications are well known in the art, see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science, 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Sproat, B. *European Patent Application* 92110298.4 which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.

By "sufficient complementarity" is meant that sufficient base pairing occurs so that the R-loop base pair structure can be formed under the appropriate conditions to cause transcription of the enzymatic nucleic acid. Those in the art will recognize routine tests by which such sufficient base pairs can be determined. In general, between about 15 - 80 bases is sufficient in this invention.

By "physiological condition" is meant the condition in the cell or tissue to be targeted by the first nucleic acid molecule, although the R-loop complex may be formed under many other conditions. One example is use of a standard physiological saline at 37°C, but it is simply desirable in this invention that the R-loop structure exists to some extent at the site of action so that the expression of the desired nucleic acid will be achieved at that site of action. While it is preferred that the R-loop structure be stable under

- those conditions, even a minimal amount of formation of the R-loop structure to cause expression will be sufficient. Those in the art will recognize that measurement of such expression is readily achieved, especially in the absence of any promoter or leader sequence on the first nucleic acid molecule (Daube and von Hippel, 1992 Science 258, 1320).
- 5 Such expression can thus only be achieved if an R-loop structure is truly formed with the second nucleic acid. If a promoter or leader sequence is provided, then it is preferred that the R-loop be formed at a site distant from those regions so that transcription is enhanced.
- 10 In a related aspect, the invention features a method for introduction of ribonucleic acid within a cell or tissue by forming an R-loop base-paired structure (as described above) with the first nucleic acid molecule lacking any promoter region or transcription termination signal such that once expression is initiated it will continue until the first nucleic acid is degraded.
- 15 In another related aspect, the invention features a method in which the second nucleic acid is provided with a localization factor, such as a protein, *e.g.*, an antibody, transferin, a nuclear localization peptide, or folate, or other such compounds well known in the art, which will aid in targeting the R-loop complex to a desired cell or tissue.
- 20 In preferred embodiments, the first nucleic acid is a plasmid, *e.g.*, one without a promoter or a transcription termination signal; the second nucleic acid is of length between about 40-200 bases and is formed of ribonucleotides at a majority of positions; and the second nucleic acid is covalently bonded with a ligand such as a nucleic acid, protein, peptide,
- 25 lipid, carbohydrate, cellular receptor, nuclear localization factor, or is attached to maleimide or a thiol group: the first nucleic acid is an expression plasmid lacking a promoter able to express a desired gene, *e.g.*, it is a double-stranded molecule formed with a majority of deoxyribonucleic acids; the R-loop complex is a RNA/DNA heteroduplex;
- 30 no promoter or leader region is provided in the first nucleic acid; and the R-loop is adapted to prevent nucleosome assembly and is designed to aid recruitment of cellular transcription machinery.

In other preferred embodiments, the first nucleic acid encodes one or more enzymatic nucleic acids, *e.g.*, it is formed with a plurality of

intramolecular and intermolecular cleaving enzymatic nucleic acids to allow release of therapeutic enzymatic nucleic acid *in vivo*.

In a further related aspect, the invention features a complex of the above first nucleic acid molecules and second nucleic acid molecules.

5 R-loop complex

An R-loop complex is designed to provide a non-integrating plasmid so that, when an RNA polymerase binds to the plasmid, transcription is continuous until the plasmid is degraded. This is achieved by hybridizing an RNA molecule, 40 to 200 nucleotides in length, to a DNA expression
10 plasmid resulting in an R-loop structure (see figure 106). This RNA, when conjugated with a ligand that binds to a cell surface receptor, triggers internalization of the plasmid/RNA-ligand complex. Formation of R-loops in general is described by DeWet, 1987 Methods in Enzymol. 145, 235; Neuwald et al., 1977 J. Virol. 21,1019; and Meyer et al., 1986 J. UIt. Mol.
15 Str. Res. 96, 187. Thus, those in the art can readily design complexes of this invention following the teachings of the art.

Promoters placed in retroviral genomes have not always behaved as planned in that the additional promoter will serve as a stop signal or reverses the direction of the polymerase. Applicant was told that creation
20 of an R-loop between the promoter and the reporter gene increased the transfection efficiency. Incubation of an RNA molecule with a double-stranded DNA molecule, containing a region of complementarity with the RNA will result in the formation of a stable RNA-DNA hetroduplex and the DNA strand that has a sequence identical to the RNA will be displaced into
25 a loop-like structure called the R-loop. This displacement of DNA strand occurs because an RNA-DNA duplex is more stable compared to a DNA-DNA duplex. Applicant was also told that an 80 nt long RNA was used to generate a R-loop structure in a plasmid encoding the β -galactosidase gene. The R-loop was initiated either in the promoter region or in the
30 leader sequence. Plasmids containing an R-loop structure were microinjected into the cytoplasm of COS cells and the gene expression was assayed. R-loop formation in the promoter region of the plasmid inhibited expression of the gene. RNA that hybridized to the leader sequence between the promoter and the gene, or directly to the first 80
35 nucleotides of the mRNA increased the expression levels 8-10 fold. The

proposed mechanism is that R-loop formation prevents nucleosome assembly, thus making the DNA more accessible for transcription. Alternatively, the R-loop may resemble a RNA primer promoting either DNA replication or transcription (Daube and von Hippel, 1992, supra).

- 5 One of the salient features of this invention is to generate R-loops in expression vectors of choice and introduce them into cells to achieve enhanced expression from the expression vector. The presence of an R-loop may aid in the recruitment of cellular transcription machinery. Once an RNA polymerase binds to the plasmid and initiates transcription, the
10 process will continue until a termination signal is reached, or the plasmid is degraded.

- This invention will increase the expression of ribozymes inside a cell. The idea is to construct a plasmid with no transcription termination signal, such that a transcript-containing multiple ribozyme units can be
15 generated. In order to liberate unit length ribozymes, self-processing ribozymes can be cloned downstream of each therapeutic ribozyme (see figure 107) as described by Draper *supra*.

Ligand Targeting

- Another salient feature of this invention is that the RNA used to
20 generate R-loop structures can be covalently linked to a ligand (nucleic acid, proteins, peptides, lipids, carbohydrates, *etc.*). Specific ligands can be chosen such that the ligand can bind selectively to a desired cell surface receptor. This ligand-receptor interaction will help internalize a plasmid containing an R-loop. Thus, RNA is used to attach the ligand to the
25 DNA such that localization of the gene to certain regions of the cell is achieved. One of several methods can be used to attach a ligand to RNA. This includes the incorporation of deoxythymidine containing a 6 carbon spacer having a terminal primary amine into the RNA (see figure 108). This amino group can be directly derivatized with the ligand, such as folate (Lee and Low, 1994 J. Biol. Chem. 269, 3198-3204). The RNA containing a 6
30 carbon spacer with a terminal amine group is mixed with folate and the mixture is reacted with activators like 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC). This reaction should be carried out in the presence of 1-Hydroxybenzotriazole hydrate (HOBT) to prevent
35 any undesirable side reactions.

The RNA can also be derivatized with a heterobifunctional crosslinking agent (or linker) like succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB). The SMPB introduces a maleimide into the RNA. This maleimide can then react with a thiol moiety either in a peptide or in a protein. Thiols can also be introduced into proteins or peptides that lack naturally occurring thiols using succinylacetylthioacetate. The amino linker can be attached at the 5' end or 3' end of the RNA. The RNA can also contain a series of nucleotides that do not hybridize to the DNA and extend the linker away from the RNA/DNA complex, thus increasing the accessibility of the ligand for its receptor and not interfering with the hybridization. These techniques can be used to link peptides such as nuclear localization signal (NLS) peptides (Lanford et al., 1984 Cell 37, 801-813; Kalderon et al., 1984 Cell 39, 499-509; Goldfarb et al., 1986 Nature 322, 641-644) and/or proteins like the transferrin (Curiel et al., 1991 Proc. Natl. Acad. Sci. USA 88, 8850-8854; Wagner et al., 1992 Proc. Natl. Acad. Sci. USA 89, 6099-6103; Giulio et al., 1994 Cell. Signal. 6, 83-90) to the ends of R-loop forming RNA in order to facilitate the uptake and localization of the R-loop-DNA complex. To link a protein to the ends of R-loop forming RNA, an intrinsic thiol can be used to react with the maleimide or the thiols can be introduced into the protein itself using either iminothiolate or succinimidyl acetyl thioacetate (SATA; Duncan et al., 1983 Anal. Biochem 132, 68). The SATA requires an additional deprotection step using 0.5 M hydroxylamine.

In addition liposomes can be used to cause an R-loop complex to be delivered to an appropriate intracellular site by techniques well known in the art. For example, pH-sensitive liposomes (Connor and Huang, 1986 Cancer Res. 46, 3431-3435) can be used to facilitate DNA transfection.

Calcium phosphate mediated or electroporation-mediated delivery of the R-loop complex into desired cells can also be readily accomplished.

30 In vitro Selection

In vitro selection strategies can be used to select nucleic acids that a) can form stable R-loops b) selectively bind to specific cell surface receptors. These nucleic acids can then be covalently linked to each other. This will help internalize the R-loop-containing plasmid efficiently using receptor-mediated endocytosis. The *in vitro* selection (evolution) strategy is

similar to approaches developed by Joyce (Beaudry and Joyce, 1992 Science 257, 635-641; Joyce, 1992 Scientific American 267, 90-97) and Szostak (Bartel and Szostak, 1993 Science 261:1411-1418; Szostak, 1993 TIBS 17, 89-93). Briefly, a random pool of nucleic acids is synthesized wherein each member contains two domains: a) one domain consists of a region with defined (known) nucleotide sequence; b) the second domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to bind to its target (a specific region of the double strand DNA), 2) complimentary DNA (cDNA) synthesis and PCR amplification of molecules selected for their affinity to form R-loop and/or their ability to bind to a specific receptor, 3) introduction of a restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides represented at every position within the random region) or the degeneracy can be partial (Beaudry and Joyce, 1992 Science 257, 635-641). In this invention, the degenerate domain is flanked by regions containing known sequences. This random library of nucleic acids is incubated under conditions that ensure equilibrium binding to either double-stranded DNA or cell surface receptor. Following incubation, nucleic acids are converted into complementary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with desired characteristics can be separated from the rest of the population of nucleic acids by using a variety of methods (Joyce, 1992 supra). The desired pool of nucleic acids can then be carried through subsequent rounds of selection to enrich the population with the most desired traits. These molecules are then cloned in to appropriate vectors. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

Other embodiments are within the following claims.

TABLE ICharacteristics of Ribozymes**Group I Introns**

Size: ~200 to >1000 nucleotides.

Requires a U in the target sequence immediately 5' of the cleavage site.

Binds 4-6 nucleotides at 5' side of cleavage site.

Over 75 known members of this class. Found in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.

RNAseP RNA (M1 RNA)

Size: ~290 to 400 nucleotides.

RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.

Roughly 10 known members of this group all are bacterial in origin.

Hammerhead Ribozyme

Size: ~13 to 40 nucleotides.

Requires the target sequence UH immediately 5' of the cleavage site.

Binds a variable number nucleotides on both sides of the cleavage site.

14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figures 1 and 2)

Hairpin Ribozyme

Size: ~50 nucleotides.

Requires the target sequence GUC immediately 3' of the cleavage site.

Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent (Figure 3).

Hepatitis Delta Virus (HDV) Ribozyme

Size: 50 - 60 nucleotides (at present).

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Only 1 known member of this class. Found in human HDV (Figure 4).

***Neurospora* VS RNA Ribozyme**

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.
Sequence requirements not fully determined.
Binding sites and structural requirements not fully determined. Only 1
known member of this class. Found in *Neurospora* VS RNA (Figure 5).

Table 2
Human ICAM HH Target sequence

nt. Position	Target Sequences	nt. Position	Target Sequences
11	CCCCAGU C GACGCUG	386	ACCGUGU A CUGGACU
23	CUGAGCU C CUCUGCU	394	CUGGACU C CAGAACG
26	AGCUCCU C UGCUACU	420	CACCCCU C CUCUCU
31	CUCUGCU A CUCAGAG	425	CUCCCCU C UUGGCAG
34	UGCUACU C AGAGUUG	427	CCCCCU U GGCAGCC
40	UCAGAGU U GCAACCU	450	AGAACCU U ACCCUAC
48	GCAACCU C AGCCUUG	451	GAAACCU A CCUACG
54	UCAGCCU C GCUADGG	456	UUAACCU A CGCUGCC
58	CCUUGCU A UGGCUCC	495	CCAACCU C ACCGUGG
64	UADGGCU C CCAGCAG	510	UGCUGCU C CGUGGGG
96	CCGCACU C CUGGUCC	564	CUGAGGU C ACCACCA
102	UCCUGGU C CUGCUUG	592	GAGAGAU C ACCADGG
108	UCCUGCU C GGGGCTC	607	AGCCAAU U UCUCGUG
115	CGGGGCU C UGUUCCC	608	GCCAAU U CUCGUGC
119	GCUCUGU U CCCAGGA	609	CCAAU U UCGUGCC
120	CUCUGUU C CCAGGAC	611	AAUUCU C GUGGCGC
146	CAGACAU C UGUGUCC	656	GAGCUGU U UGAGAAC
152	UCUGUGU C CCCCUC	657	AGCUGU U GAGACA
158	UCCCCU C AAAAGUC	668	AACACCU C GGCCCCC
165	CAAAAGU C AUCCUGC	677	GCCCCU A CCAGCTC
168	AAGUCAU C CCGCCCC	684	ACCAGCU C CAGACCU
185	GGAGGCU C CGUGCUG	692	CAGACCU U UGUUCUG
209	AGCACCU C CUGUGAC	693	AGACCU U GUCCUGC
227	CCCAAGU U GUUGGGC	696	CCUUGU C CUGCCAG
230	AAGUUGU U GGGCAUA	709	AGCGACU C CCCCACA
237	UGGGCAU A GAGACCC	720	CACAACU U GUCAGCC
248	ACCCCGU U GCCUAAA	723	AACUUGU C AGCCCCC
253	GUUGOCU A AAAAGGA	735	CCCGGGU C CUGAGAG
263	AAGGAGU U GCUCCUG	738	GGGUCCU A GAGGUGG
267	AGUUGCU C CUGOCUG	765	CCGUGGU C UGUUCCC
293	AAGGUGU A UGAACUG	769	GGUCUGU U CCCCUGA
319	AGAAGAU A GCCAAC	770	GUCUGU C CCGGAC
335	AUGUGCU A UUCAAC	785	GGGUGU U CCCAGUC
337	GUGCUAU U CAAACUG	786	GGCUGU C CCAGUCU
338	UGCUAUU C AAACUGC	792	UCCAGU C UCGGAGG
359	GGGCAGU C AACAGCU	794	CCAGUCU C GGAGGCC
367	AACAGCU A AAACCUU	807	CCAGGU C CACUUGG
374	AAAACCU U CCUCACC	833	CAGAGGU U GAACCCC
375	AAACCUU C CUCACCG	846	CCACAGU C ACCUADG
378	CCUCCU C ACCGUGU	851	GUCACCU A UGGCAAC

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863	AACGACU C CUGCUUG	1408	UCGAGAU C UUGAGGG
866	GACUCCU U CUUGGOC	1410	GAGAUU U GAGGGCA
867	ACUCCUU C UUGGOC	1421	GGCACCU A CCUCUGU
869	UCCUUCU C GGCCAAG	1425	CCUACCU C UGUUGGG
881	AAGGCCU C AGUCAGU	1429	CCUCUGU C GGGCCAG
885	CCUCAGU C AGUGUGA	1444	GAGCACU C AAGGGGA
923	GUGCAGU A AUACUGG	1455	GGGAGGU C ACCCGCG
936	CAGUAAU A CUGGGGA	1482	AUGUGCU C UCCCCC
978	UGACCAU C UACAGCU	1484	GUGCUU C CCCCCG
980	ACCAUCU A CAGCUUU	1493	CCCCGU A UGAGAUU
986	UACAGCU U UCUGGCG	1500	AUGAGAU U GUCADCA
987	ACAGCUU U CCGGCGC	1503	AGAUUGU C AUCAUCA
988	CAGCUUU C CGGCGCC	1506	UUGUCAU C AUCACUG
1005	ACUGAUU U CUGAGA	1509	UCAUCAU C ACUGUGG
1006	CGUGAUU C UGAGAA	1518	CUUGGU A GCAGCG
1023	CAGAGGU C UCAGAAG	1530	CGCAGU C AUAAAGG
1025	GAGGUCU C AGAAGGG	1533	CAGUCAU A AUGGGCA
1066	CCACCU A GAGOC	1551	CAGGCU C AGCAAGU
1092	AUGGGGU U CCAGOC	1559	AGCAGU A CCUCU
1093	UGGGGU C CAGOC	1563	CGUACCU C UAUAA
1125	CCAGCU C CUGCUGA	1565	UACUCU A UAACCG
1163	CGCAGU U CUOCGC	1567	CCUCU A ACGOCA
1164	GCAGCU C UCUCGU	1584	GGAGAU C AAGAAU
1166	AGCUUCU C CUGCUU	1592	AAGAAU A CAGACU
1172	UCCUGCU C UGCAACC	1599	ACAGACU A CAACAGG
1200	GCCAGCU U AUACACA	1651	CAGGCU C CUGAAC
1201	CCAGCU A UACACA	1661	UGAACCU A UCCCGG
1203	AGCUU A CACAAGA	1663	AACCU C CCGGAC
1227	GGGAGCU U CGUGUCC	1678	AGGCCU C UUCUUG
1228	GGAGCU C GUGUCCU	1680	GGCUCU U CCUGGC
1233	UUGUGU C CUGUUG	1681	GCCUCU C CUUGGC
1238	GUCCUGU A UGGCCCC	1684	UCUUCU C GGCUUC
1264	GAGGGAU U GUCCGGG	1690	UGGCCU U CCUAU
1267	GGAUGU C CGGAAA	1691	CGGCCU C CCUAU
1294	AGAAAU U CCAGCA	1696	UUCUCAU A UUGGUGG
1295	GAAAU C CCAGCAG	1698	CCUAU U GGUGCA
1306	GCAGACU C CAUGUG	1737	AAGACAU A UGCCADG
1321	CCAGGU U GGGGAA	1750	UGCAGU A CAUCUAC
1334	AACCAU U GCGGAG	1756	UACACCU A CCGGCC
1344	CCGAGCU C AAGUGUC	1787	AGGCCAU U GUCCUA
1351	CAAGUGU C UAAAGGA	1790	GCAUGU C CUCAGUC
1353	AGUGUCU A AAGGADG	1793	UUGUCCU C AGUCAGA
1366	UGSCACU U UCCACU	1797	CCUCAGU C AGAUACA
1367	GGCACU U CCACUG	1802	GUCAGAU A CAACAGC
1368	GCACUU C CCACUGC	1812	ACAGCAU U UGGGGCC
1380	UGCCAU C GGGGAU	1813	CAGCAU U GGGGCCA
1388	GGGGAU C AGUGACU	1825	CCADGGU A CCUGCAC
1398	UGACUGU C ACUCGAG	1837	CACACU A AACACU
1402	UGUCACU C GAGAUU	1845	AAACACU A GGCCAG

1856	CACGCCAU C UGADCCG	2189	UADUUUAD U GAGGCGC
1861	AUCUGAU C UGUGAGC	2196	UGAGUGU C UUUUADG
1865	GADCCGU A GUCACAU	2198	AGUGUCU U UUADGUA
1868	CUGUAGU C ACADGAC	2199	GUGUCUU U UADGUAG
1877	CAUGACU A AGCCAAG	2200	UGUCUUU U ADGUAGG
1901	CAAGACU C AAGACAU	2201	GUCUUUU A UGUAGGC
1912	ACADGAU U GADGGAU	2205	UUUADGU A GGCUAAA
1922	UGGAUGU U AAAGUCU	2210	GUGAGCU A AAUGAAC
1923	GGAUUUU A AAGUCUA	2220	UGRACAU A GGCCUCU
1928	UUAAAGU C UAGCCCG	2224	CADAGGU C UCUGGOC
1930	AAAGUCU A GCCUGAU	2226	UAGGUCU C UGGCCUC
1964	GAGACAU A GCCCCAC	2233	CUGGOCU C ACGGAGC
1983	AGGACAU A CACCGG	2242	CGGAGCU C CCGGCC
1996	GGGAAAU A CUGAAAC	2248	UCCAGU C CAUGUCA
2005	UGAAACU U GCUGCCU	2254	UCCAUU C ACAUACA
2013	GCUGCCU A UUGGGUA	2259	GUCACAU U CAAGGUC
2015	UGCCUAD U GGGUADG	2260	UCACAUU C AAGGUCA
2020	AUUGGGU A UGCGAG	2266	UCAAGGU C ACCAGGU
2039	ACAGACU U ACAGAAG	2274	ACCAGGU A CAGUGU
2040	CAGACUU A CAGAAGA	2279	GUACAGU U GUACAGG
2057	UGGCCCU C CAUAGAC	2282	CAGUUGU A CAGGUUG
2061	CCUCCAU A GACADGU	2288	UACAGGU U GUACACU
2071	CAUGUGU A GCAUCUA	2291	AGGUUGU A CACUGCA
2076	GUAGCAU C AAAACAC	2321	AAAAGAU C AAADGGG
2097	CCACACU U CCUGAGG	2338	UGGGACU U CUCAUUG
2098	CACACUU C CUGAGGG	2339	GGGACUU C UCADUGG
2115	GCCAGCU U GGGCACU	2341	GACUUCU C AUUGGCC
2128	CUGCUGU C UACUGAC	2344	UUCUACU U GGCCAAC
2130	GCUGUCU A CUGACCC	2358	CCUGCCU U UCCCCAG
2145	CAACCCU U GADGADA	2359	CUGCCUU U CCCCAGA
2152	UGADGAU A UGUADUU	2360	UGCCUUU C CCGAGAA
2156	GAUADGU A UUUADUC	2376	GAGUGAU U UUUUADU
2158	UADGUAD U UAUUCAU	2377	AGUGAUU U UUCUADU
2159	AUGUADU U AUUCAUU	2378	GUGADUU U UCUADCG
2160	UGUADUU A UUCAUUU	2379	UGADUUU U CUADCGG
2162	UADUUAD U CAUUUGU	2380	GADUUUU C UADGGGC
2163	AUUUADU C AUUGGUU	2382	UUUUUCU A UCGGCAC
2166	UADUCAU U UGUUADU	2384	UUUCUAD C GGCACAA
2167	AUUCAUU U GUUADUU	2399	AAGCACU A UADGGAC
2170	CADUUGU U ADUUUAC	2401	GCACUAD A UGGACUG
2171	AUUUGUU A UUUUACC	2411	GACUGGU A AUGGUUC
2173	UUGUUAD U UUACCAG	2417	UAAUGGU U CACAGGU
2174	UGUUADU U UACCAGC	2418	AAUGGUU C ACAGGUU
2175	GUUADUU U ACCAGCU	2425	CACAGGU U CAGAGAU
2176	UUADUUU A CCAGCUA	2426	ACAGGUU C AGAGAUU
2183	ACCAGCU A UUUADUG	2433	CAGAGAU U ACCCAGU
2185	CAGCUAU U UAUUGAG	2434	AGAGAUU A CCGAGUG
2186	AGCUADU U ADUGAGU	2448	GAGGCCU U AUUCUCU
2187	GUUADUU A UUGAGUG	2449	AGGCCUU A UUCUCUC

2451	GCCUUAU	U	CCUCCCU	2750	UAUGUGU	A	GACAAGC
2452	CCUUAUU	C	CCUCCCU	2759	ACAAGCU	C	UCGCUCU
2455	UAUCCCU	C	CCUCCCU	2761	AAGCUCU	C	GCUCUGU
2459	CCUCCCU	U	CCUCCCA	2765	UCUCCCU	C	UGUCACC
2460	CUCCCUU	C	CCUCCAA	2769	GCUCUGU	C	ACCCAGG
2479	GACACCU	U	UGUAGGC	2797	GUGCAAU	C	AUGGUUC
2480	ACACCUU	U	GUUAGCC	2803	UCADGGU	U	CACUGCA
2483	CCUUGGU	U	AGCCACC	2804	CADGGUU	C	ACUGCAG
2484	CUUUGUU	A	GCCACCU	2813	CUGCAGU	C	UUGACCU
2492	GCCACCU	C	CCUCCCU	2815	GCAGUCU	U	GACCUUU
2504	CCCACAU	A	CAUUCUU	2821	UUGACCU	U	UUGGGCU
2508	CAUACAU	U	UCUGCCA	2822	UGACCUU	U	UGGGCUU
2509	AUACAUU	U	CUGCCAG	2823	GACCUUU	U	GGGCUCA
2510	UACAUUU	C	UGCCAGU	2829	UUGGGCU	C	AAGUGAU
2520	CCAGUGU	U	CACAAUG	2837	AAGUGAU	C	CUCCCAC
2521	CAGUGUU	C	ACAADGA	2840	UGAUCCU	C	CCACCUU
2533	UGACACU	C	AGGGGUC	2847	CCACCUU	C	AGCCUUC
2540	CAGGGGU	C	ADGUCUG	2853	UCAGCCU	C	CUGAGUA
2545	GUCAUGU	C	UGGACAU	2860	CCUGAGU	A	GCUGGGA
2568	AGGGAUU	A	UGUCCAA	2872	GGACCAU	A	GGUCCAC
2579	CCAAGCU	A	UGUCCUG	2877	AUAGCCU	C	ACAACAC
2585	UAUGCCU	U	GUUCCUU	2899	GGCAAAU	U	UGAUUUU
2588	GCCUUGU	C	CUUUGUU	2900	GCAAAUU	U	GAUUUUU
2591	UUGUCCU	C	UUGUCCU	2904	ADUUGAU	U	UUUUUUU
2593	GUUCCUU	U	GUUCCUU	2905	UUUGAUU	U	UUUUUUU
2596	CUUUGUU	C	CUUUGUG	2906	UUGAUUU	U	UUUUUUU
2601	GUUCCUU	U	UGCAUUU	2907	UGAUUUU	U	UUUUUUU
2602	UCCUGUU	U	GCAUUUC	2908	GAUUUUU	U	UUUUUUU
2607	UUUGCAU	U	UCACUGG	2909	ADUUUUU	U	UUUUUUU
2608	UUGCAUU	U	CACUGGG	2910	UUUUUUU	U	UUUUUUU
2609	UGCAUUU	C	ACUGGGA	2911	UUUUUUU	U	UUUUUUU
2620	GGGAGCU	U	GCAUUAU	2912	UUUUUUU	U	UUUUUUC
2626	UUGCACU	A	UUGCAGC	2913	UUUUUUU	U	UUUUUCA
2628	GCACUAU	U	GCAGCUC	2914	UUUUUUU	U	UUUUCAG
2635	UGCAGCU	C	CAGUUUC	2915	UUUUUUU	U	UUUCAGA
2640	CUCCAGU	U	UCCUGCA	2916	UUUUUUU	U	UUCAGAG
2641	UCCAGUU	U	CCUGCAG	2917	UUUUUUU	U	UCAGAGA
2642	CCAGUUU	C	CUGCAGU	2918	UUUUUUU	U	CAGAGAC
2653	CAGUGAU	C	AGGGUCC	2919	UUUUUUU	C	AGAGACG
2659	UCAGGGU	C	CUGCAAG	2931	ACGGGGU	C	UUGCAAC
2689	CCAAGGU	A	UUGGAGG	2933	GGGGUCU	C	GCAACAU
2691	AAGGUUU	U	GGAGGAC	2941	GCAACAU	U	GCCAGA
2700	GAGGACU	C	CCUCCCA	2951	CCAGACU	U	CCUUGUU
2704	ACUCCCU	C	CCAGCUU	2952	CAGACUU	C	CUUUGUG
2711	CCCAGCU	U	UGGAAGG	2955	ACUUCUU	U	UGUGUUA
2712	CCAGCUU	U	GGAAGGG	2956	CUUCCUU	U	GUGUAG
2721	GAAGGGU	C	AUCCGCG	2961	UUUGUGU	U	AGUUAUU
2724	GGGUCAU	C	CGUGUGU	2962	UUGUGUU	A	GUUAAUA
2744	UGUGUGU	A	UGUGUAG	2965	UGUUGAU	U	AUUAAAG

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2966	GUAAGUU A AUAAGC
2969	AGUUAUU A AAGCUU
2975	UAAAGCU U UCUAAC
2976	AAAGCUU U CUCAACU
2977	AAGCUUU C UCAACUG
2979	GCUUUCU C AACUGCC

Table 3

Mouse ICAM HH Target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
11	CCGugGU C aCCGUG	367	AAugGCU u cAAOCcg
23	CaGuGgU u CUUGCU	374	gAAgCCU U CCUGcCC
26	uGgUuCU C UGCUcCU	375	AAgCCU C CUgcCCc
31	CUUGCU c CUCCaCa	378	CuacCaU C ACCGUGU
34	UuUcaU a AGgGUcG	386	ACCGUGU A uUcGuU
40	gCacAcU U GuAgCCU	394	CcGGACU u ucGGuCu
48	aggAOCU C AGCCUGG	420	CACaCuU C CCCcCcg
54	UggGCCU C GuGADGG	425	CaCCCCU C ccaGCAG
58	CaUgcCU u UaGCUCC	427	CagCCU c aGCAGug
64	cAcccCU C CCAGCAG	450	AGgAOCU c ACCCUgC
96	CucugCU C CUGGcCC	451	GAAaCCU u uCCUuuG
102	UgCcaGU a CUGCUgG	456	UUAOCU c aGCcaCu
108	cuCUGCU C cuGGCCc	495	CuAcCaU C ACCGUGu
115	uGGuuCU C UGcUCCu	510	UGCUGCU C CGUGGGG
119	GgaaUGU c aCCAGGA	564	CUcAGGU a uCcAuCc
120	CUUGCU C CugGccC	592	GAAAGAU C ACaugGG
146	CAGuGgU C cGcuUCC	607	AGCCAAU U UCUCaUG
152	UCUGUGU C agCCaCu	608	GCCAAU U CUCzUGC
158	UCCugU u AAAAacC	609	CCAAU U UCaUGCC
165	CagAAGU u gUuuUGC	611	AAUUCU C aUGCCGC
168	AAGcCuU C CUGCCCC	656	zAGCUGU U UGAGcug
185	GUUGGgU C CGUGCaG	657	AGCUGU U GAGcugA
209	gcCAcuU C CUUGgC	668	cgagCCU a GGCCaCC
227	CagAAGU U GUUuuGC	677	GaCCuCU A CCAGCcu
230	AAGUUGU U uuGCucc	684	uuCAGCU C CgGuCCU
237	UGuGcuU u GAGAaCu	692	CgGACuU U cGauCUu
248	AaCCCaU c uCCUAAA	693	AGgaCcU c acCCUGC
253	ccUGCCU A AggAaGA	696	CCUgGuU C CUGCCuc
263	AgGGuuU c uCUaCUG	709	gGCGgCU C CaCCuCA
267	AGggGCU C CUGCCUa	720	uACAACTU U uUCAGCu
293	AAGcUGU u UGAgCUG	723	AACUuuU C AGCuCCg
319	AGgAGAU A cugAgCC	735	aCCaGaU C CUgGAGa
335	cUGUGCU u UgzgAAC	738	uGGgCCU c GuGaUGG
337	GUcCaAU U CACACUG	765	CaGUcGU C cGcUuCC
338	aGCUgUU u gAgCUGa	769	GGcCUGU U uCCUGcc
359	GuGCAGU C guCcGCU	770	uUuUGcU C CCUGGAa
785	GGcCUGU U uCCuGcC	1353	AGUGggU c gAaGgUG
786	GcCUGUU u CCuGcCU	1366	UaaCAGU c UaCaACTU
792	UggagGU C UCGGAaG	1367	aGCACcU c CCCACcu
794	CugGgCU u GGAGaCu	1368	GuACUgU a CCACUcu
807	CuCgGaU a uACCUGG	1380	UGCCCAU C GGGGugg
833	CAaAGcU c GAcacCC	1388	GGaGAcU C AGUGgCU
846	CCcugGU C ACCguUG	1398	UGgCUGU C ACagaAc
851	GagAOCU c UacCAGC	1402	UGUgcU u GAGAaCU

863	AgCcACU u CcUCUgG	1408	gCGAGAU C ggGgaGG
866	GAagCCU U CcuGcCC	1410	GAGgUCU c GgaaGgg
867	AuUCgUU u cCGGagA	1421	ccCACCU A CuUuUGU
869	UCuUcCU C augCAAG	1425	aCUgCCU u gGUaGaG
881	AuGGCUU C AacCcGU	1429	uCUUaU u GccCUuG
885	CCUugGU a gagGUGA	1444	GAaggCU C AgGaGGA
933	cUauAaU c ADuCTGG	1455	GGaAuGU C ACCaGga
936	uAaUcAU u CCGGuGc	1482	AgUUGuU u UgCuCCC
978	UaACagU C UACAaCU	1484	cUGuUCU u CCuCaUG
980	ACagUCU A CAaCUUU	1493	CuguGcU u UGAGAac
986	UACAaCU U DuCaGCU	1500	ADGAaAU c aUggUCc
987	ACAaCUU U uCaGCUc	1503	gGAcUaU a ADCAUuc
988	CAaCUUU u CaGCUCC	1506	UUaUguU u AUaACcG
1005	ACcaGAU c CUGgaGA	1509	cuAcCAU C ACcGUGu
1006	uGaGAgU C UGggGAA	1518	ucaUGGU c cCAGgOG
1023	ugGAGGU C UCgGAAG	1530	QuauAaU C AUucUGG
1025	GAGGUUU C gGAAGGG	1533	ugGUCAU u gUGGGCc
1066	CCACuCU c aAaauAA	1551	CAUGCCU u AGCAgcU
1092	AcuGGaU c uCAGgCC	1559	AGCACcU c CCaaccU
1093	UGGaccU u CAGCCaA	1563	CuUAugU u UADAACC
1125	CCCAaCU C uUcuUGA	1565	UAugUuU A UAACCGC
1163	CGaAGCU U CUuuUGC	1567	ugUuUAU A ACCGCCA
1164	GaAGCUU C DuuUGCU	1584	GaAAGAU C AgGAuAU
1166	AGCUUCU u uUGCUU	1592	AgGAuAU A CAaguUA
1172	UCCUGuU u aaaAACC	1599	ACAaguU A CAgaAGG
1200	cuCuGCU c cUcCACA	1651	CcCaCCU C CCUGAgC
1201	gCuGCUU u UgaACag	1661	gaAAccU u UCCuuuG
1203	AcuUUuU u CACcAGu	1663	AAccUuU C CUuuGAa
1227	GGuAcaU a CGUGUgC	1678	AGGaCCU C agCCUgG
1228	GaAGCUU C uDuUgCU	1680	aGCCaCU U CCUCuGg
1233	UUCGUuU C CgGagaG	1681	GCCaCUU C CUUUGgC
1238	GUgCUGU A UGGuCCu	1684	aCUUCCU C uGgCUgu
1264	GAaGGgU c GUgCaaG	1690	cCGGaCU U uCgAUcU
1267	uGagaGU C uGGGgAA	1691	CGGaCUU u CgAUcUU
1294	AGgAgAU a CugAGCc	1696	UgCCCAU c ggGGUGG
1295	GAggggU C uCAGCAG	1698	CggAUUAU a ccUGGag
1306	GCAGACU C ugAaaUG	1737	gAGACcU c UaCCAgc
1321	gaAGGCU c aGGaGgA	1750	gGCGGCU c CAccUca
1334	AAccCAU c uCCuaAa	1756	gAagCCU u CCuGCCC
1344	auGAGCU C gAGaGUg	1787	gaGaCAU U GUCCcCA
1351	ugAaUGU a UAAguuA	1790	GCAUUGU u CUcuaau
1793	UgGUCCU C gGcuGGA	2173	UUagagU U UUAOCAG
1797	CacCAGU C AcAUaAa	2174	UagagUU U UAACCAGC
1802	acCAGAU c CuggAGa	2175	agagUUU U ACCAGCU
1812	ACuGgAU c UcaGGCC	2176	gagUUUU A CCAGCUA
1813	CAGCAUU U acccuCA	2183	ACCAGCU A UUUADUG
1825	CCAoGcU A CCUcugC	2185	CAGCUAU U UAUGAG
1837	CAugCCU u uAgCuCc	2186	AGCUAUU U AUUGAGU
1845	cgAgcCU A GGCCACc	2187	GCUAUUU A UUGAGUa

1856	CggaCuU u cGADCUu	2189	UAGUUAU U GAGUacC
1861	AcaUGAU a UccAGUa	2196	caAcUcU u cUUGADG
1865	cAcuUGU A GcCuCAG	2198	gcaGcCU c UADGGu
1868	CaccAGU C ACAUaAa	2199	GccUCCU a UgUuUAu
1877	CAUGcCU u AGCagcu	2200	UcUuccU c ADGcAaG
1901	uAAaACU C AAGggAc	2201	aagUUUU A UGUcGGC
1912	AuAUagU a GADcagU	2205	UUUADGU c GGCcugA
1922	UGaADGU a uAAGUua	2210	GgAGaCU c AgUGgcu
1923	uGADGcU c AgGUaUc	2220	cuggCAU u GuUCCU
1928	UUAgAGU u UuaCCaG	2224	CucAGGU a UCcauCC
1930	AgAGUuU u aCCaGcU	2226	UgGaUcU C aGGCCgC
1964	GAGACAU u GuCCCa	2233	CUGaCCU C cuGGAGg
1983	AGGauAU A CAAGUua	2242	uGGAGCU a gCgGaCC
1996	aGGAgAU A CUGagcC	2248	UauCcaU C CAUccCA
2005	UGgAgCU a GCGaCc	2254	UCCaauU C ACAcUgA
2013	GCUauuU A UUGaGUA	2259	aUCACAU U CAcGGUg
2015	UGCCcAU c GGGgugG	2260	UCACAUU C AcGGUgc
2020	ggUGGuU c UuCUAG	2266	ggAAuGU C ACCAGGa
2039	gCuGgCU a gCAGAgG	2274	ACCAGaU c CuGgaGa
2040	CuGACcU c CuGgAGg	2279	GaAggGU c GUgCAaG
2057	UGcuCCU C CAcAucc	2282	aAGcUGU u ugaGcUG
2061	CuaCCAU c acCgUGU	2288	UauAaGU U aUggcCU
2071	CACuUGU A GCcUcAg	2291	caGUgGU u CuUGCU
2076	GUAGCcU C AgAgCua	2321	gAAAGAU C AcAUggG
2097	CaACuCU U CuUGauG	2338	UGaGACU c CUgccUG
2098	CACACU C CcccCcG	2339	GaaACcU u UCcUuUG
2115	GCCAGCU c GGaggau	2341	GACcUCU a ccaGcCu
2128	CaGCUaU u UAUUGAg	2344	UUucgAU c uuCCAgC
2130	cCUGUuU c CUGcCuC	2358	CCcagCU c UCagCAG
2145	CAAcuCU U cuUGAUg	2359	CUGCuUU U gaaCAGA
2152	UauUaAU u UagAgUU	2360	aaCCUUU C UuuGAA
2156	uugAUGU A UUUAUa	2376	agGUGgU U cUUCUga
2158	gADGUAU U UADUaAU	2377	gGUGgUU c UUCUgag
2159	AUGUAUU U AUUaAUU	2378	agGgUUU c UCUAcuG
2160	UGUAUUU A UUaAUUU	2379	UGcUUUU c ucAUaaG
2162	UADUUAU U aADUUag	2380	aAgUUUU a UgUcGGC
2163	AUGUAUU u ADUaaUU	2382	aUUcUCU A UuGcCcC
2166	acUUCAU U cucUADU	2384	aUcCagU a GaCACA
2167	AUguAUU U aUUAaUU	2399	AAaCACU A UgUGGAC
2170	uADUUaU U AaJUUAg	2401	aagCUgU u UGagCUG
2171	AgUUGUU u UgcUcCC	2411	uACUGGU c AgGaUgC
2417	gAADGGU a CAuAcGU	2691	AAuGUcU c cEAGGcC
2418	AcUGGaU C uCAGGcc	2700	GAAgCcu u CCUGCCc
2425	CAugGGU c gAGgGuU	2704	gacCuCU a CCAGCcU
2426	AuuaaUU u AGAGuUU	2711	CCCAGCU c UcagcaG
2433	uAGAGuU U uaCCAGc	2712	gagGucU c GEAAGGG
2434	AGAGuUU u aCCAGcu	2721	GAAGGGU C gUgCaazG
2448	GAAGCCU U ccUgCcC	2724	GGuaCAU a CGuGUGc
2449	AaGCCUU c cUgCcCC	2744	gGUGgGU c cGUGcAG

2451	GCCUguU U CCUGCCU	2750	UAUuUaU u GAguAcc
2452	CCUguUU C CUgCCUc	2759	cCggaCU u UCGaUCU
2455	gAagCCU u CCUGCCC	2761	AgGacCU C aCccUGc
2459	CCaCaCU U CCCCCCc	2765	UUUUGCCU C UGcCgCu
2460	CaCaCUU C CCCCCcg	2769	agUCCGU C AaaCAGG
2479	GAgAOCU c UaccAGC	2797	aUGaAAU C AUGGUcC
2480	uCACCGU U GUgAuCC	2803	UCAUGGU c CcagGCg
2483	CCaaUGU c AGCCACC	2804	ggUGGgU C cgUGCAG
2484	CUUuuUU c aCCAguc	2813	CUcCgGU C cUGACCc
2492	agCACCU C CCCACCu	2815	aCAGUCU a cAaCUUU
2504	CCCACcU A CuUUUGU	2821	cUGACCU c cUGGagg
2508	uAUcCAU c caUcCCA	2822	gGAgCcU c cGGaCUu
2509	uUAgAgU U uUaCCAG	2823	ugCCUUU a GcuCcCA
2510	UAgAgUU u UaCCAGc	2829	cUGGaCU a uAaUcAU
2520	CuuuUGU U CcCAADG	2837	AgGUGgU u CUuCuGa
2521	CAGcaUU u ACccUcA	2840	UGAgacu C CugCCUg
2533	UGAugCU C AGguaUC	2847	CCaAugU C AGCCaCC
2540	CAGCaGU C cgcUGUG	2853	gCAGCCU C uUauGUu
2545	GUgcUGU a UGGuCcU	2860	gCcaAGU A aCUGuGA
2568	guGaAgU c UGuCaAA	2872	GGACCUU c aGCCaAg
2579	auAAGuU A UGgCcUG	2877	uUccGCU a cCAuCAC
2585	cugGCaU U GUuCUUU	2899	cGgAcuU U cGAUcUU
2588	GCaUUGU u CUUaaU	2900	uuAAuUU a GAgUUUU
2591	UGGUuCU C UgcUCCU	2904	AcUUcAU U cUcUaUU
2593	cUUUUUU U GcuCUGc	2905	cUUcAUU c UcUaUUG
2596	CUuUUGU u CccaaUG	2906	UUGAUgU a UUuaUUa
2601	acCgUGU a UUcGUUU	2907	UGuaUUU a UUaaUUU
2602	UCCaGcU a cCAUccC	2908	GAagcUU c UUUUgcU
2607	cUcGgAU a UaccUGG	2909	AgcUUcU U UUgcUcU
2608	caGCAgU c CgCUGuG	2910	UgUaUUU a UUaaUUU
2609	gGaAUgU C ACcaGGA	2911	UgUaUUU a UUaaUUU
2620	aGGAcCU c aCcCUgc	2912	UUgUUcU c UaaUgUC
2626	UUuGgaU c UUcCAGC	2913	UUUcUcU a cUggUCA
2628	GCACacU U GuAGCcu	2914	UgcUUUU c UcaUaAG
2635	UUcAGCU C CgGUccu	2915	aUUUaUU a aUUuAGA
2640	ggCCuGU U UCCUGCc	2916	UaUUcgU U UcCgGAG
2641	cCCAGcU c uCaGCAG	2917	aUUcgUU U cCgGAGA
2642	CCuGUUU C CUGCcuC	2918	UUcgUUU c CgGAGAg
2653	uAcUGgU C AGGaUgC	2919	UUcUcaU a AGgGuCG
2659	gaAGGGU C gUGCAAG	2931	ugGaGGU C UCGgAAg
2689	CuAAuGU c UccGAGG	2933	GaGGUCU C GgAAggg
2941	GagACAU U GuCCcCA		
2951	CCAagCU a CCUcUGc		
2952	CAGcagU C CgcUGUG		
2955	AgUgaCU c UGUGUcA		
2956	uUUCCUU U GaaUcAa		
2961	UcUGUGU c AGccAcU		
2962	aUGUaUU u aUUAADu		
2965	UuUgAaU c AAUAAG		

2966 GcUgGcU A gcAgAGg
2969 AaUcAAU A AAGuUUU
2975 UAgAGuU U UacCAgC
2976 gAgGgUU U CUcUACU
2977 AAGCUgU u UgAgCUG
2979 uCaUUUU C uAuUGCC

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Table 4
Human ICAM HH Ribozyme Sequences

nt. Position	Ribozyme Sequence
11	CAGCGUC CUGAUGAGGCCGAAAGGCCGAA ACUGGGG
23	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCUCAG
26	AGUAGCA CUGAUGAGGCCGAAAGGCCGAA AGGAGCU
31	CUUCUGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
34	CAACUCU CUGAUGAGGCCGAAAGGCCGAA AGUAGCA
40	AGGUUGC CUGAUGAGGCCGAAAGGCCGAA ACUCUGA
48	CGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUUGC
54	CCAUAGC CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
58	GGAGCCA CUGAUGAGGCCGAAAGGCCGAA AGOGAGG
64	CUGCUGG CUGAUGAGGCCGAAAGGCCGAA AGCCAUA
96	GGACCAG CUGAUGAGGCCGAAAGGCCGAA AGUGCGG
102	CGAGCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGA
108	GAGCCOC CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
115	GGGAACA CUGAUGAGGCCGAAAGGCCGAA AGCCCCG
119	UOCUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGAGC
120	GUCCUGG CUGAUGAGGCCGAAAGGCCGAA AACAGAG
146	GGACACA CUGAUGAGGCCGAAAGGCCGAA AUGUCUG
152	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA ACACAGA
158	GACUUUU CUGAUGAGGCCGAAAGGCCGAA AGGGGGA
165	GCAGGAU CUGAUGAGGCCGAAAGGCCGAA ACUUUUG
168	GGGGCAG CUGAUGAGGCCGAAAGGCCGAA AUGACUU
185	CAGCAGC CUGAUGAGGCCGAAAGGCCGAA AGCCUCC
209	GUACAGC CUGAUGAGGCCGAAAGGCCGAA AGGUGCU
227	GCCCCAC CUGAUGAGGCCGAAAGGCCGAA ACUUGGG
230	UADGCCC CUGAUGAGGCCGAAAGGCCGAA ACAACUU
237	GGGUCUC CUGAUGAGGCCGAAAGGCCGAA AUGCCCA
248	UUUAGGC CUGAUGAGGCCGAAAGGCCGAA ACGGGGU
253	UCCUUUU CUGAUGAGGCCGAAAGGCCGAA AGGCAAC
263	CAGGAGC CUGAUGAGGCCGAAAGGCCGAA ACUCCUU
267	CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAACU
293	CAGUUCA CUGAUGAGGCCGAAAGGCCGAA ACACCUU
319	GGUUGGC CUGAUGAGGCCGAAAGGCCGAA AUCUUCU
335	GUUUGAA CUGAUGAGGCCGAAAGGCCGAA AGCACAU
337	CAGUUUG CUGAUGAGGCCGAAAGGCCGAA AUAGCAC
338	GCAGUUU CUGAUGAGGCCGAAAGGCCGAA AAUAGCA
359	AGCUGUU CUGAUGAGGCCGAAAGGCCGAA ACUGCCC
367	AAGGUUU CUGAUGAGGCCGAAAGGCCGAA AGCUGUU
374	GGUGAGG CUGAUGAGGCCGAAAGGCCGAA AGGUUUU
375	CGGUGAG CUGAUGAGGCCGAAAGGCCGAA AAGGUUU
378	ACACGGU CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
386	AGUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACGGU
394	CGUUCUG CUGAUGAGGCCGAAAGGCCGAA AGUCCAG
420	AAGAGGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUG
425	CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AGGGGAG

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427	GGCUGCC	CUGAUGAGGCOGAAAGGCOGAA	AGAGGGG
450	GUAAGGU	CUGAUGAGGCOGAAAGGCOGAA	AGGUUCU
451	CGUAGGG	CUGAUGAGGCOGAAAGGCOGAA	AAGGUUC
456	GGCAGCG	CUGAUGAGGCOGAAAGGCOGAA	AGGGUAA
495	CCACGGU	CUGAUGAGGCOGAAAGGCOGAA	AGGUUGG
510	CCCCACG	CUGAUGAGGCOGAAAGGCOGAA	AGCAGCA
564	UGGUUGU	CUGAUGAGGCOGAAAGGCOGAA	ACCUACG
592	CCAUGGU	CUGAUGAGGCOGAAAGGCOGAA	AUCUCUC
607	CACGAGA	CUGAUGAGGCOGAAAGGCOGAA	AUUGGCU
608	GCAAGAG	CUGAUGAGGCOGAAAGGCOGAA	AAUUGGC
609	GGACCA	CUGAUGAGGCOGAAAGGCOGAA	AAAUUGG
611	GCGGCAC	CUGAUGAGGCOGAAAGGCOGAA	AGAAAUU
656	GUUCUCA	CUGAUGAGGCOGAAAGGCOGAA	ACAGCUC
657	UGUUCUC	CUGAUGAGGCOGAAAGGCOGAA	AACAGCU
668	GGGGGCC	CUGAUGAGGCOGAAAGGCOGAA	AGGUGUU
677	GAGCUGG	CUGAUGAGGCOGAAAGGCOGAA	AGGGGGC
684	AGGUUUG	CUGAUGAGGCOGAAAGGCOGAA	AGCUGGU
692	CAGGACA	CUGAUGAGGCOGAAAGGCOGAA	AGGUCUG
693	GCAGGAC	CUGAUGAGGCOGAAAGGCOGAA	AAGGUUC
696	CUGGCAG	CUGAUGAGGCOGAAAGGCOGAA	ACAAAGG
709	UGUGGGG	CUGAUGAGGCOGAAAGGCOGAA	AGUUGCU
720	GGCUGAC	CUGAUGAGGCOGAAAGGCOGAA	AGUUGUG
723	GGGGGCU	CUGAUGAGGCOGAAAGGCOGAA	ACAAGUU
735	CCUCUAG	CUGAUGAGGCOGAAAGGCOGAA	ACCCGGG
738	CCACCUU	CUGAUGAGGCOGAAAGGCOGAA	AGGACCC
765	GGGAACA	CUGAUGAGGCOGAAAGGCOGAA	ACCAAGG
769	UCCAGGG	CUGAUGAGGCOGAAAGGCOGAA	ACAGACC
770	GUCCAGG	CUGAUGAGGCOGAAAGGCOGAA	AACAGAC
785	GACUGGG	CUGAUGAGGCOGAAAGGCOGAA	ACAGCCC
786	AGACUGG	CUGAUGAGGCOGAAAGGCOGAA	AACAGCC
792	CCUCOGA	CUGAUGAGGCOGAAAGGCOGAA	ACUGGGA
794	GGCUCU	CUGAUGAGGCOGAAAGGCOGAA	AGACUGG
807	CCAGGUG	CUGAUGAGGCOGAAAGGCOGAA	ACCUGGG
833	GGGGUUC	CUGAUGAGGCOGAAAGGCOGAA	ACCUUCG
846	CAUAGGU	CUGAUGAGGCOGAAAGGCOGAA	ACUGUGG
851	GUUGCCA	CUGAUGAGGCOGAAAGGCOGAA	AGGUGAC
863	CGAGAAG	CUGAUGAGGCOGAAAGGCOGAA	AGUUGUU
866	GGCOGAG	CUGAUGAGGCOGAAAGGCOGAA	AGGAGUC
867	UGGCOGA	CUGAUGAGGCOGAAAGGCOGAA	AAGGAGU
869	CUUGGOC	CUGAUGAGGCOGAAAGGCOGAA	AGAAGGA
881	ACUGACU	CUGAUGAGGCOGAAAGGCOGAA	AGGOCUU
885	UCACACU	CUGAUGAGGCOGAAAGGCOGAA	ACUGAGG
933	CCAGUAT	CUGAUGAGGCOGAAAGGCOGAA	ACUGCAC
936	UCCCCAG	CUGAUGAGGCOGAAAGGCOGAA	AUUACUG
978	AGCUGUA	CUGAUGAGGCOGAAAGGCOGAA	AUGGUCA
980	AAAGCUG	CUGAUGAGGCOGAAAGGCOGAA	AGAUUGU
986	CGCOGGA	CUGAUGAGGCOGAAAGGCOGAA	AGCUGUA
987	GCGCOGG	CUGAUGAGGCOGAAAGGCOGAA	AAGCUGU
988	GGCGCCG	CUGAUGAGGCOGAAAGGCOGAA	AAAGCUG

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1005	UUGUCAG	CUGAUGAGGCGGAAAGGCGGAA	AUUCAGU
1006	UUCGUCA	CUGAUGAGGCGGAAAGGCGGAA	AADUACG
1023	CUUCUGA	CUGAUGAGGCGGAAAGGCGGAA	ACCUUCU
1025	CCUUCU	CUGAUGAGGCGGAAAGGCGGAA	AGACCUU
1066	UGGUCUC	CUGAUGAGGCGGAAAGGCGGAA	AGGUGUG
1092	GGGCUUG	CUGAUGAGGCGGAAAGGCGGAA	ACCCCAU
1093	UGGUCUG	CUGAUGAGGCGGAAAGGCGGAA	AACCCCA
1125	UCAGCAG	CUGAUGAGGCGGAAAGGCGGAA	AGCUUGG
1163	GCAGGAG	CUGAUGAGGCGGAAAGGCGGAA	AGCUUGG
1164	AGCAGGA	CUGAUGAGGCGGAAAGGCGGAA	AAGCUUG
1166	AGAGCAG	CUGAUGAGGCGGAAAGGCGGAA	AGAAGCU
1172	GGUUGCA	CUGAUGAGGCGGAAAGGCGGAA	AGCAGGA
1200	UGUGUAU	CUGAUGAGGCGGAAAGGCGGAA	AGCUUGG
1201	UUGUGUA	CUGAUGAGGCGGAAAGGCGGAA	AAGCUUG
1203	UCUUGUG	CUGAUGAGGCGGAAAGGCGGAA	AUAAGCU
1227	GGACACG	CUGAUGAGGCGGAAAGGCGGAA	AGCUUCC
1228	AGGACAC	CUGAUGAGGCGGAAAGGCGGAA	AAGCUUCC
1233	CAUACAG	CUGAUGAGGCGGAAAGGCGGAA	ACAAGAA
1238	GGGGCCA	CUGAUGAGGCGGAAAGGCGGAA	ACAGGAC
1264	CCCGGAC	CUGAUGAGGCGGAAAGGCGGAA	AUCCCUU
1267	UUUCCCG	CUGAUGAGGCGGAAAGGCGGAA	ACAADCC
1294	UGCUGGG	CUGAUGAGGCGGAAAGGCGGAA	AUUUUCU
1295	CUGCUGG	CUGAUGAGGCGGAAAGGCGGAA	AUUUUCU
1306	CACAUUG	CUGAUGAGGCGGAAAGGCGGAA	AGUCUGG
1321	UUCCCCC	CUGAUGAGGCGGAAAGGCGGAA	AGCCUGG
1334	CUCCGGC	CUGAUGAGGCGGAAAGGCGGAA	ADGGGUU
1344	GACACUU	CUGAUGAGGCGGAAAGGCGGAA	AGCUUGG
1351	UCCUUUA	CUGAUGAGGCGGAAAGGCGGAA	ACACUUG
1353	CAUCCUU	CUGAUGAGGCGGAAAGGCGGAA	AGACACU
1366	AGUGGGA	CUGAUGAGGCGGAAAGGCGGAA	AGUGCCA
1367	CAGUGGG	CUGAUGAGGCGGAAAGGCGGAA	AAGUGCC
1368	GCAGUGG	CUGAUGAGGCGGAAAGGCGGAA	AAAGUGC
1380	AUUCCCC	CUGAUGAGGCGGAAAGGCGGAA	AUGGGCA
1388	AGUCACU	CUGAUGAGGCGGAAAGGCGGAA	AUUCCCC
1398	CUCCAGU	CUGAUGAGGCGGAAAGGCGGAA	ACAGUCA
1402	AGAUCUC	CUGAUGAGGCGGAAAGGCGGAA	AGUGACA
1408	CCCUCAA	CUGAUGAGGCGGAAAGGCGGAA	AUCUUGA
1410	UGCCCUU	CUGAUGAGGCGGAAAGGCGGAA	AGAUCUC
1421	ACAGAGG	CUGAUGAGGCGGAAAGGCGGAA	AGGUGCC
1425	CCCGACA	CUGAUGAGGCGGAAAGGCGGAA	AGGUAGG
1429	CUGGCC	CUGAUGAGGCGGAAAGGCGGAA	ACAGAGG
1444	UCCCCUU	CUGAUGAGGCGGAAAGGCGGAA	AGUGCUC
1455	CGCGGGU	CUGAUGAGGCGGAAAGGCGGAA	ACUCCCC
1482	GGGGGGA	CUGAUGAGGCGGAAAGGCGGAA	AGCACAU
1484	CCGGGGG	CUGAUGAGGCGGAAAGGCGGAA	AGAGCAC
1493	AADCUCA	CUGAUGAGGCGGAAAGGCGGAA	ACCGGGG
1500	UGAUGAC	CUGAUGAGGCGGAAAGGCGGAA	AUCUCAU
1503	UGAUGAU	CUGAUGAGGCGGAAAGGCGGAA	ACAUCU
1506	CAGUGAU	CUGAUGAGGCGGAAAGGCGGAA	AUGACAA

1509	CCACAGU	CUGAUGAGGCOGAAAGGCOGAA	ADGADGA
1518	CGGCTGC	CUGAUGAGGCOGAAAGGCOGAA	ACCACAG
1530	CCAUUAU	CUGAUGAGGCOGAAAGGCOGAA	ACUGCGG
1533	UGCCCAU	CUGAUGAGGCOGAAAGGCOGAA	AUGACUG
1551	ACGUGCU	CUGAUGAGGCOGAAAGGCOGAA	AGGOCUG
1559	AUAGAGG	CUGAUGAGGCOGAAAGGCOGAA	ACGUGCU
1563	GGUUAUA	CUGAUGAGGCOGAAAGGCOGAA	AGGUACG
1565	GCGGUUA	CUGAUGAGGCOGAAAGGCOGAA	AGAGGUA
1567	UGGCGGU	CUGAUGAGGCOGAAAGGCOGAA	AUAGAGG
1584	AUUUCUU	CUGAUGAGGCOGAAAGGCOGAA	AUCUUCU
1592	UAGUCUG	CUGAUGAGGCOGAAAGGCOGAA	AUUUCUU
1599	CCUGUUG	CUGAUGAGGCOGAAAGGCOGAA	AGUCUGU
1651	GUUCAGG	CUGAUGAGGCOGAAAGGCOGAA	AGGOGUG
1661	CCCGGGA	CUGAUGAGGCOGAAAGGCOGAA	AGGUUCA
1663	GUCCCGG	CUGAUGAGGCOGAAAGGCOGAA	AUAGGUU
1678	CGAGGAA	CUGAUGAGGCOGAAAGGCOGAA	AGGOCUU
1680	GCGGAGG	CUGAUGAGGCOGAAAGGCOGAA	AGAGGCC
1681	GGCGGAG	CUGAUGAGGCOGAAAGGCOGAA	AAGAGGC
1684	GAAGGCC	CUGAUGAGGCOGAAAGGCOGAA	AGGAGGA
1690	AUAUGGG	CUGAUGAGGCOGAAAGGCOGAA	AGGCOGA
1691	AAUAUGG	CUGAUGAGGCOGAAAGGCOGAA	AAGGCOG
1696	CCACCAA	CUGAUGAGGCOGAAAGGCOGAA	AUGGGAA
1698	UGCCACC	CUGAUGAGGCOGAAAGGCOGAA	AUAUGGG
1737	CAUGGCA	CUGAUGAGGCOGAAAGGCOGAA	AUGUCUU
1750	GUAGGUG	CUGAUGAGGCOGAAAGGCOGAA	AGUCUGA
1756	GGGCGGG	CUGAUGAGGCOGAAAGGCOGAA	AGGUGUA
1787	UGAGGAC	CUGAUGAGGCOGAAAGGCOGAA	AUGOCUU
1790	GACUGAG	CUGAUGAGGCOGAAAGGCOGAA	ACRAUGC
1793	UCUGACT	CUGAUGAGGCOGAAAGGCOGAA	AGGACAA
1797	UGUAUCU	CUGAUGAGGCOGAAAGGCOGAA	ACUGAGG
1802	GCUGUUG	CUGAUGAGGCOGAAAGGCOGAA	AUCUGAC
1812	GGCCCCA	CUGAUGAGGCOGAAAGGCOGAA	AUGCUGU
1813	UGGCCCC	CUGAUGAGGCOGAAAGGCOGAA	AAUGCTG
1825	GUGCAGG	CUGAUGAGGCOGAAAGGCOGAA	ACCAUGG
1837	AGUGUUU	CUGAUGAGGCOGAAAGGCOGAA	AGGUGUG
1845	CGUGGCC	CUGAUGAGGCOGAAAGGCOGAA	AGUGUUU
1856	CAGAUCA	CUGAUGAGGCOGAAAGGCOGAA	ADGOGUG
1861	GACUACA	CUGAUGAGGCOGAAAGGCOGAA	AUCAGAU
1865	AUGUGAC	CUGAUGAGGCOGAAAGGCOGAA	ACAGAUU
1868	GUCAUGU	CUGAUGAGGCOGAAAGGCOGAA	ACUACAG
1877	CUUGGCU	CUGAUGAGGCOGAAAGGCOGAA	AGUCADG
1901	AUGUCUU	CUGAUGAGGCOGAAAGGCOGAA	AGUCUUG
1912	AUCCAUU	CUGAUGAGGCOGAAAGGCOGAA	AUCAUGU
1922	AGACUUU	CUGAUGAGGCOGAAAGGCOGAA	ACAUCCA
1923	UAGACUU	CUGAUGAGGCOGAAAGGCOGAA	AACAUCC
1928	CAGGCUA	CUGAUGAGGCOGAAAGGCOGAA	ACUUAUA
1930	AUCAGGC	CUGAUGAGGCOGAAAGGCOGAA	AGACUUU
1964	GUGGGGC	CUGAUGAGGCOGAAAGGCOGAA	AUGUCUC
1983	CCAGUUG	CUGAUGAGGCOGAAAGGCOGAA	AUGUCCU

1996	GUUUCAG	CUGAUGAGGCGGAAAGGCGGAA	AUUUCCC
2005	AGGCAGC	CUGAUGAGGCGGAAAGGCGGAA	AGUUUCA
2013	UACCCAA	CUGAUGAGGCGGAAAGGCGGAA	AGGCAGC
2015	CATAACC	CUGAUGAGGCGGAAAGGCGGAA	AUAGGCA
2020	CUCAGCA	CUGAUGAGGCGGAAAGGCGGAA	ACCCAAU
2039	CUUCUGU	CUGAUGAGGCGGAAAGGCGGAA	AGUCUGU
2040	UCUUCUG	CUGAUGAGGCGGAAAGGCGGAA	AAGUCUG
2057	GUCUADG	CUGAUGAGGCGGAAAGGCGGAA	AGGGCCA
2061	ACAUUGC	CUGAUGAGGCGGAAAGGCGGAA	AUGGAGG
2071	UUGAUGC	CUGAUGAGGCGGAAAGGCGGAA	ACACADG
2076	GUGUUUU	CUGAUGAGGCGGAAAGGCGGAA	AUGCUAC
2097	CGUCAGG	CUGAUGAGGCGGAAAGGCGGAA	AGUGUGG
2098	COGUCAG	CUGAUGAGGCGGAAAGGCGGAA	AAGUGUG
2115	AGUGCCC	CUGAUGAGGCGGAAAGGCGGAA	AGCUGGC
2128	GUCAGUA	CUGAUGAGGCGGAAAGGCGGAA	ACAGCAG
2130	GGGUCAG	CUGAUGAGGCGGAAAGGCGGAA	AGACAGC
2145	UAUCAUC	CUGAUGAGGCGGAAAGGCGGAA	AGGGUUG
2152	AAAUACA	CUGAUGAGGCGGAAAGGCGGAA	AUCAUCA
2156	GAUAATA	CUGAUGAGGCGGAAAGGCGGAA	ACAUADC
2158	AUGAADA	CUGAUGAGGCGGAAAGGCGGAA	AUACADA
2159	AAUGAAU	CUGAUGAGGCGGAAAGGCGGAA	AAUACAU
2160	AAAUCAA	CUGAUGAGGCGGAAAGGCGGAA	AAAUACA
2162	ACAAAUU	CUGAUGAGGCGGAAAGGCGGAA	AUAAAUU
2163	AACAAAU	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
2166	AAUAACA	CUGAUGAGGCGGAAAGGCGGAA	AUGAAUA
2167	AAAUAAC	CUGAUGAGGCGGAAAGGCGGAA	AAUGAAU
2170	GUAAAAU	CUGAUGAGGCGGAAAGGCGGAA	ACAAAUU
2171	GGUAAA	CUGAUGAGGCGGAAAGGCGGAA	AACAAAU
2173	CUGGUAA	CUGAUGAGGCGGAAAGGCGGAA	AUAACAA
2174	GCUGGUA	CUGAUGAGGCGGAAAGGCGGAA	AAUAACA
2175	AGCUGGU	CUGAUGAGGCGGAAAGGCGGAA	AAAUAAC
2176	UAGCUGG	CUGAUGAGGCGGAAAGGCGGAA	AAAUAUA
2183	CAUAATA	CUGAUGAGGCGGAAAGGCGGAA	AGCUGGU
2185	CUCAATA	CUGAUGAGGCGGAAAGGCGGAA	AUAGCUG
2186	ACUCAAU	CUGAUGAGGCGGAAAGGCGGAA	AAUAGCU
2187	CACUCAA	CUGAUGAGGCGGAAAGGCGGAA	AAAUAGC
2189	GACACUC	CUGAUGAGGCGGAAAGGCGGAA	AUAUAUA
2196	CAUAAAA	CUGAUGAGGCGGAAAGGCGGAA	ACACUCA
2198	UACAUAA	CUGAUGAGGCGGAAAGGCGGAA	AGACACU
2199	CUACATA	CUGAUGAGGCGGAAAGGCGGAA	AAGACAC
2200	CCUACAU	CUGAUGAGGCGGAAAGGCGGAA	AAAGACA
2201	GCCUACA	CUGAUGAGGCGGAAAGGCGGAA	AAAAGAC
2205	UUUAGOC	CUGAUGAGGCGGAAAGGCGGAA	ACAUAAA
2210	GUUCADU	CUGAUGAGGCGGAAAGGCGGAA	AGCCUAC
2220	AGAGACC	CUGAUGAGGCGGAAAGGCGGAA	AUGUUCA
2224	GGCCAGA	CUGAUGAGGCGGAAAGGCGGAA	ACCUADG
2226	GAGGCOA	CUGAUGAGGCGGAAAGGCGGAA	AGACCUA
2233	GCUCOGU	CUGAUGAGGCGGAAAGGCGGAA	AGGOCAG
2242	GGACUGG	CUGAUGAGGCGGAAAGGCGGAA	AGCUCOG

2248	UGACAU	UGAUGAGGCGAAAGGCGAA	ACUGGGA
2254	UGAAUGU	UGAUGAGGCGAAAGGCGAA	ACADGGA
2259	GAOCUUG	UGAUGAGGCGAAAGGCGAA	AUGUGAC
2260	UGACCUU	UGAUGAGGCGAAAGGCGAA	AADUGGA
2266	ACCUUGU	UGAUGAGGCGAAAGGCGAA	ACCUUGA
2274	ACAACUG	UGAUGAGGCGAAAGGCGAA	ACCUUGU
2279	CCUGUAC	UGAUGAGGCGAAAGGCGAA	ACUGUAC
2282	CAACUUG	UGAUGAGGCGAAAGGCGAA	ACAACTG
2288	AGUGUAC	UGAUGAGGCGAAAGGCGAA	ACCUUGA
2291	UGCAGUG	UGAUGAGGCGAAAGGCGAA	ACAAOCU
2321	CCCAUUU	UGAUGAGGCGAAAGGCGAA	AUCUUUU
2338	CAUGAG	UGAUGAGGCGAAAGGCGAA	AGUCCCA
2339	CCAAUGA	UGAUGAGGCGAAAGGCGAA	AAGUCCC
2341	GGCCAAU	UGAUGAGGCGAAAGGCGAA	AGAAGUC
2344	GUUGGCC	UGAUGAGGCGAAAGGCGAA	AUGAGAA
2358	CUGGGGA	UGAUGAGGCGAAAGGCGAA	AGGCAGG
2359	UCUGGGG	UGAUGAGGCGAAAGGCGAA	AAGGCAG
2360	UUCUGGG	UGAUGAGGCGAAAGGCGAA	AAAGGCA
2376	AUAGAAA	UGAUGAGGCGAAAGGCGAA	AUCACUC
2377	GAUAGAA	UGAUGAGGCGAAAGGCGAA	AADCACT
2378	CGAUAGA	UGAUGAGGCGAAAGGCGAA	AAADCAC
2379	COGAUAG	UGAUGAGGCGAAAGGCGAA	AAAADCA
2380	GCGAUA	UGAUGAGGCGAAAGGCGAA	AAAAADC
2382	GUGCCGA	UGAUGAGGCGAAAGGCGAA	AGAAAAA
2384	UUGUGOC	UGAUGAGGCGAAAGGCGAA	AUAGAAA
2399	GUCCAU	UGAUGAGGCGAAAGGCGAA	AGUGCUU
2401	CAGUCCA	UGAUGAGGCGAAAGGCGAA	AUAGUGC
2411	GAACCAU	UGAUGAGGCGAAAGGCGAA	ACCAGUC
2417	ACCUUGU	UGAUGAGGCGAAAGGCGAA	ACCAUUA
2418	AAOCUUG	UGAUGAGGCGAAAGGCGAA	AAOCUUG
2425	AUCUCUG	UGAUGAGGCGAAAGGCGAA	ACCUUGU
2426	AAUCUCU	UGAUGAGGCGAAAGGCGAA	AAOCUUG
2433	ACUGGGU	UGAUGAGGCGAAAGGCGAA	AUCUCUG
2434	CACUGGG	UGAUGAGGCGAAAGGCGAA	AAUCUCU
2448	GAGGAUU	UGAUGAGGCGAAAGGCGAA	AGGCCUC
2449	GGAGGAA	UGAUGAGGCGAAAGGCGAA	AAGGCCU
2451	AGGGAGG	UGAUGAGGCGAAAGGCGAA	AUAAGGC
2452	AAGGGAG	UGAUGAGGCGAAAGGCGAA	AAUAAGG
2455	GGGAAGG	UGAUGAGGCGAAAGGCGAA	AGGAUUA
2459	UGGGGGG	UGAUGAGGCGAAAGGCGAA	AGGGAGG
2460	UUGGGGG	UGAUGAGGCGAAAGGCGAA	AAGGGAG
2479	GCUAACA	UGAUGAGGCGAAAGGCGAA	AGGUGUC
2480	GGCUAAC	UGAUGAGGCGAAAGGCGAA	AAGGUGU
2483	GGUGGCU	UGAUGAGGCGAAAGGCGAA	ACAAAGG
2484	AGGUGGC	UGAUGAGGCGAAAGGCGAA	AACAAAG
2492	GGGUGGG	UGAUGAGGCGAAAGGCGAA	AGGUGGC
2504	AGAAADG	UGAUGAGGCGAAAGGCGAA	AUGUGGG
2508	UGGCAGA	UGAUGAGGCGAAAGGCGAA	AUGUAUG
2509	CUGGCAG	UGAUGAGGCGAAAGGCGAA	AAUGUAU

2510	ACUGGCA	CUGAUGAGGCOGAAAGGCOGAA	AAADGUA
2520	CAUUGUG	CUGAUGAGGCOGAAAGGCOGAA	ACACUGG
2521	UCADUGU	CUGAUGAGGCOGAAAGGCOGAA	AACACUG
2533	GACCGCU	CUGAUGAGGCOGAAAGGCOGAA	AGUGUCA
2540	CAGACAU	CUGAUGAGGCOGAAAGGCOGAA	ACCGCUG
2545	AUGUCCA	CUGAUGAGGCOGAAAGGCOGAA	ACAUGAC
2568	UUGGGCA	CUGAUGAGGCOGAAAGGCOGAA	AUOCCCU
2579	CAAGGCA	CUGAUGAGGCOGAAAGGCOGAA	AGCUUGG
2585	AGAGGAC	CUGAUGAGGCOGAAAGGCOGAA	AGGCAUA
2588	ACAAGAG	CUGAUGAGGCOGAAAGGCOGAA	ACAAGGC
2591	AGGACAA	CUGAUGAGGCOGAAAGGCOGAA	AGGACAA
2593	ACAGGAC	CUGAUGAGGCOGAAAGGCOGAA	AGAGGAC
2596	CAAAACAG	CUGAUGAGGCOGAAAGGCOGAA	ACAAGAG
2601	AAADGCA	CUGAUGAGGCOGAAAGGCOGAA	ACAGGAC
2602	GAAAUCC	CUGAUGAGGCOGAAAGGCOGAA	AACAGGA
2607	CCAGUGA	CUGAUGAGGCOGAAAGGCOGAA	AUGCAAA
2608	CCCAGUG	CUGAUGAGGCOGAAAGGCOGAA	AAUGCAA
2609	UCCACAU	CUGAUGAGGCOGAAAGGCOGAA	AAADGCA
2620	AUAGUGC	CUGAUGAGGCOGAAAGGCOGAA	AGCUOCC
2626	GCUGCAA	CUGAUGAGGCOGAAAGGCOGAA	AGUGCAA
2628	GAGCUGC	CUGAUGAGGCOGAAAGGCOGAA	AUAGUGC
2635	GAAACUG	CUGAUGAGGCOGAAAGGCOGAA	AGCUCCA
2640	UGCAGGA	CUGAUGAGGCOGAAAGGCOGAA	ACUGGAG
2641	CUGCAGG	CUGAUGAGGCOGAAAGGCOGAA	AACUGGA
2642	ACUGCAG	CUGAUGAGGCOGAAAGGCOGAA	AAACUGG
2653	GGACCCU	CUGAUGAGGCOGAAAGGCOGAA	AUCACUG
2659	CUUGCAG	CUGAUGAGGCOGAAAGGCOGAA	ACCCUGA
2689	CCUCCAA	CUGAUGAGGCOGAAAGGCOGAA	ACCUUGG
2691	GUCCUCC	CUGAUGAGGCOGAAAGGCOGAA	AUAOCUU
2700	UGGGAGG	CUGAUGAGGCOGAAAGGCOGAA	AGUCCUC
2704	AAGCUGG	CUGAUGAGGCOGAAAGGCOGAA	AGGGAGU
2711	CCUUGCA	CUGAUGAGGCOGAAAGGCOGAA	AGCUUGG
2712	CCCUUCC	CUGAUGAGGCOGAAAGGCOGAA	AAGCUUG
2721	CGGGGAU	CUGAUGAGGCOGAAAGGCOGAA	ACCCUUC
2724	ACACGGG	CUGAUGAGGCOGAAAGGCOGAA	AUGAOCU
2744	CUACACA	CUGAUGAGGCOGAAAGGCOGAA	ACACACA
2750	GCUUGUC	CUGAUGAGGCOGAAAGGCOGAA	ACACATA
2759	AGAGCGA	CUGAUGAGGCOGAAAGGCOGAA	AGCUUGU
2761	ACAGAGC	CUGAUGAGGCOGAAAGGCOGAA	AGAGCUU
2765	GGUGACA	CUGAUGAGGCOGAAAGGCOGAA	AGCGAGA
2769	CCUGGGU	CUGAUGAGGCOGAAAGGCOGAA	ACAGAGC
2797	GAACCAU	CUGAUGAGGCOGAAAGGCOGAA	AUUGCAC
2803	UGCAGUG	CUGAUGAGGCOGAAAGGCOGAA	ACCAUGA
2804	CUGCAGU	CUGAUGAGGCOGAAAGGCOGAA	AACCAUG
2813	AGGUCAA	CUGAUGAGGCOGAAAGGCOGAA	ACUGCAG
2815	AAAGGUC	CUGAUGAGGCOGAAAGGCOGAA	AGACUGC
2821	AGCCCAA	CUGAUGAGGCOGAAAGGCOGAA	AGGUCAA
2822	GAGCCCA	CUGAUGAGGCOGAAAGGCOGAA	AAGGUCA
2823	UGAGCCC	CUGAUGAGGCOGAAAGGCOGAA	AAAGGUC

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2829	AUCACUU	CUGAUGAGGCOGAAAGGCOGAA	AGCOCAA
2837	GUGGGAG	CUGAUGAGGCOGAAAGGCOGAA	AUCACUU
2840	GAGGUGG	CUGAUGAGGCOGAAAGGCOGAA	AGGAUCA
2847	GGAGGCU	CUGAUGAGGCOGAAAGGCOGAA	AGGUGGG
2853	UACUCAG	CUGAUGAGGCOGAAAGGCOGAA	AGGCUGA
2860	UCCAGC	CUGAUGAGGCOGAAAGGCOGAA	ACUCAGG
2872	GUGAGCC	CUGAUGAGGCOGAAAGGCOGAA	AUGGUCC
2877	GUGUGU	CUGAUGAGGCOGAAAGGCOGAA	AGCCTAU
2899	AAADCA	CUGAUGAGGCOGAAAGGCOGAA	AUUUGCC
2900	AAAAUC	CUGAUGAGGCOGAAAGGCOGAA	AUUUGC
2904	AAAAAA	CUGAUGAGGCOGAAAGGCOGAA	AUCAAU
2905	AAAAAA	CUGAUGAGGCOGAAAGGCOGAA	AADCAA
2906	AAAAAA	CUGAUGAGGCOGAAAGGCOGAA	AAADCA
2907	AAAAAA	CUGAUGAGGCOGAAAGGCOGAA	AAAADCA
2908	AAAAAA	CUGAUGAGGCOGAAAGGCOGAA	AAAAADC
2909	AAAAAA	CUGAUGAGGCOGAAAGGCOGAA	AAAAAU
2910	AAAAAA	CUGAUGAGGCOGAAAGGCOGAA	AAAAAA
2911	AAAAAA	CUGAUGAGGCOGAAAGGCOGAA	AAAAAA
2912	GAAAAA	CUGAUGAGGCOGAAAGGCOGAA	AAAAAA
2913	UGAAAA	CUGAUGAGGCOGAAAGGCOGAA	AAAAAA
2914	CUGAAA	CUGAUGAGGCOGAAAGGCOGAA	AAAAAA
2915	UCUGAA	CUGAUGAGGCOGAAAGGCOGAA	AAAAAA
2916	CUCUGA	CUGAUGAGGCOGAAAGGCOGAA	AAAAAA
2917	UCUCUG	CUGAUGAGGCOGAAAGGCOGAA	AAAAAA
2918	GUCUCG	CUGAUGAGGCOGAAAGGCOGAA	AAAAAA
2919	CGUCUC	CUGAUGAGGCOGAAAGGCOGAA	AAAAAA
2931	GUUGCA	CUGAUGAGGCOGAAAGGCOGAA	ACCOGU
2933	AUGUUG	CUGAUGAGGCOGAAAGGCOGAA	AGACCC
2941	UCUGGC	CUGAUGAGGCOGAAAGGCOGAA	AUGUUG
2951	ACAAAG	CUGAUGAGGCOGAAAGGCOGAA	AGUCUG
2952	CACAAAG	CUGAUGAGGCOGAAAGGCOGAA	AAGUCG
2955	UAACAC	CUGAUGAGGCOGAAAGGCOGAA	AGGAAGU
2956	CUAACAC	CUGAUGAGGCOGAAAGGCOGAA	AAGGAAG
2961	AUUAACT	CUGAUGAGGCOGAAAGGCOGAA	ACACAA
2962	UAUUAC	CUGAUGAGGCOGAAAGGCOGAA	AACACAA
2965	CUUUUU	CUGAUGAGGCOGAAAGGCOGAA	ACUACA
2966	GCUUUU	CUGAUGAGGCOGAAAGGCOGAA	AACUAC
2969	AAAGCU	CUGAUGAGGCOGAAAGGCOGAA	AUUAACT
2975	GUUGAG	CUGAUGAGGCOGAAAGGCOGAA	AGCUUA
2976	AGUUGAG	CUGAUGAGGCOGAAAGGCOGAA	AAGCUU
2977	CAGUUG	CUGAUGAGGCOGAAAGGCOGAA	AAAGCU
2979	GGCAGU	CUGAUGAGGCOGAAAGGCOGAA	AGAAAGC

Table 5

Mouse ICAM HH Ribozyme Sequence
nt. Position Ribozyme Sequence

11	CAACGGU	CUGAUGAGGCOGAAAGGCOGAA	ACCAGGG
23	AGCAGAG	CUGAUGAGGCOGAAAGGCOGAA	ACCACTG
26	AGGAGCA	CUGAUGAGGCOGAAAGGCOGAA	AGAACCA
31	UGGGGAG	CUGAUGAGGCOGAAAGGCOGAA	AGCAGAG
34	CGACCCU	CUGAUGAGGCOGAAAGGCOGAA	AUGAGAA
40	AGGCUAC	CUGAUGAGGCOGAAAGGCOGAA	AGUGUGC
48	CCAGGCU	CUGAUGAGGCOGAAAGGCOGAA	AGGUCCU
54	CCAUAC	CUGAUGAGGCOGAAAGGCOGAA	AGGCCCA
58	GGAGCUA	CUGAUGAGGCOGAAAGGCOGAA	AGGCAUG
64	CUGCUGG	CUGAUGAGGCOGAAAGGCOGAA	AGGGGUG
96	GGGCCAG	CUGAUGAGGCOGAAAGGCOGAA	AGCAGAG
102	CCAGCAG	CUGAUGAGGCOGAAAGGCOGAA	ACUGGCA
108	GGGCCAG	CUGAUGAGGCOGAAAGGCOGAA	AGCAGAG
115	AGGAGCA	CUGAUGAGGCOGAAAGGCOGAA	AGAACCA
119	UCCUGGU	CUGAUGAGGCOGAAAGGCOGAA	ACAUUCC
120	GGGCCAG	CUGAUGAGGCOGAAAGGCOGAA	AGCAGAG
146	GGAAGCG	CUGAUGAGGCOGAAAGGCOGAA	ACGACTG
152	AGUGGCU	CUGAUGAGGCOGAAAGGCOGAA	ACACAGA
158	GGUUGUU	CUGAUGAGGCOGAAAGGCOGAA	AACAGGA
165	GCAAAAC	CUGAUGAGGCOGAAAGGCOGAA	ACUUCUG
168	GGGCCAG	CUGAUGAGGCOGAAAGGCOGAA	AAGGCUU
185	CUGCAGG	CUGAUGAGGCOGAAAGGCOGAA	ACCCACC
209	GCCAGAG	CUGAUGAGGCOGAAAGGCOGAA	AAGUGGC
227	GCAAAAC	CUGAUGAGGCOGAAAGGCOGAA	ACUUCUG
230	GGAGCAA	CUGAUGAGGCOGAAAGGCOGAA	ACAACTU
237	AGUUCUC	CUGAUGAGGCOGAAAGGCOGAA	AAGCACA
248	UUUAGGA	CUGAUGAGGCOGAAAGGCOGAA	AUGGGUU
253	UCUUCUU	CUGAUGAGGCOGAAAGGCOGAA	AGGCAGG
263	CAGUAGA	CUGAUGAGGCOGAAAGGCOGAA	AAACCCU
267	UAGGCAG	CUGAUGAGGCOGAAAGGCOGAA	AGCCCCU
293	CAGCUCA	CUGAUGAGGCOGAAAGGCOGAA	ACAGCUU
319	GGCUCAG	CUGAUGAGGCOGAAAGGCOGAA	AUCUCCU
335	GUUCUCA	CUGAUGAGGCOGAAAGGCOGAA	AGCACAG
337	CAGUGUG	CUGAUGAGGCOGAAAGGCOGAA	AUUGGAC
338	UCAGCUC	CUGAUGAGGCOGAAAGGCOGAA	AACAGCU
359	AGCGGAC	CUGAUGAGGCOGAAAGGCOGAA	ACUGCAC
367	CGGGUUG	CUGAUGAGGCOGAAAGGCOGAA	AGCCAUU
374	GGGCAGG	CUGAUGAGGCOGAAAGGCOGAA	AGGCUUC
375	GGGCCAG	CUGAUGAGGCOGAAAGGCOGAA	AAGGCUU
378	ACAAGGU	CUGAUGAGGCOGAAAGGCOGAA	AUGGUAG
386	AAACGAA	CUGAUGAGGCOGAAAGGCOGAA	ACAAGGU
394	AGAUCGA	CUGAUGAGGCOGAAAGGCOGAA	AGUCCGG
420	CGGGGGG	CUGAUGAGGCOGAAAGGCOGAA	AAGUGUG
425	CUGCUGG	CUGAUGAGGCOGAAAGGCOGAA	AGGGGUG

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427	CACUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGAGCUG
450	GCAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
451	CAAAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUUC
456	AGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGGUAA
495	ACACGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAG
510	CCCCACG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
564	GGAUUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUAGG
592	CCCAUGU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUCU
607	CAUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCU
608	GCAUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGC
609	GGCAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGG
611	GCGGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUU
656	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
657	UCAGCUC	CUGAUGAGGCCGAAAGGCCGAA	AACAGCU
668	GGUGGOC	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUG
677	AGGCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUC
684	AGEACCG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAA
692	AAGAUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCG
693	GCAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
696	GAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
709	UGAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCGCC
720	AGCUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUA
723	CGGAGCU	CUGAUGAGGCCGAAAGGCCGAA	AAAAGUU
735	UCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGU
738	CCAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCA
765	GGAAGCG	CUGAUGAGGCCGAAAGGCCGAA	ACGACUG
769	GGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCC
770	UUCGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAA
785	GGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCC
786	AGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGGC
792	CUUCOGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
794	AGUCUCC	CUGAUGAGGCCGAAAGGCCGAA	AGCCCCG
807	CCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCCGAG
833	GGGUGUC	CUGAUGAGGCCGAAAGGCCGAA	AGCUUUG
846	CAACGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
851	GUUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
863	CCAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGCU
866	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
867	UCUCCGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAU
869	CUUGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGA
881	ACGGGUU	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAU
885	UCACCUU	CUGAUGAGGCCGAAAGGCCGAA	ACCAAGG
933	CCAGAAU	CUGAUGAGGCCGAAAGGCCGAA	AUUAUAG
936	GCACCAG	CUGAUGAGGCCGAAAGGCCGAA	ADGADUA
978	AGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	ACUGUUA
980	AAAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGACUGU
986	AGCUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUA
987	GAACUGA	CUGAUGAGGCCGAAAGGCCGAA	AAGUUGU
988	GGAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGUUGU

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1005	UCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGU
1006	UUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCA
1023	CUUCCGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
1025	CCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACUUC
1066	UUUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAGUGG
1092	GGCCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCAGU
1093	UUGGCUU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCA
1125	UCAAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGG
1163	GCAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCG
1164	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUC
1166	AGAGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCU
1172	GGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACAGGA
1200	UGUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
1201	CUGUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGC
1203	ACUGGGU	CUGAUGAGGCCGAAAGGCCGAA	AAAAAGU
1227	GCAACAG	CUGAUGAGGCCGAAAGGCCGAA	AUGUACC
1228	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUC
1233	CUUCUUG	CUGAUGAGGCCGAAAGGCCGAA	AAACGAA
1238	AGGAACA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAC
1264	CUUGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCUUUC
1267	UUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCA
1294	GGCUUAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUU
1295	CUGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACCUUUC
1306	CAUUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUGC
1321	UCCUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCUUUC
1334	UUUAGGA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGUU
1344	CACUCUC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCAU
1351	UAACUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCA
1353	CACCUUC	CUGAUGAGGCCGAAAGGCCGAA	ACCCACU
1366	AGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	ACUGUUA
1367	AGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGCU
1368	AGAGUGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUAC
1380	CCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
1388	AGCCACU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCC
1398	GUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGCCA
1402	AGUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AAGCACA
1408	CCUCCCC	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGC
1410	CCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACUUC
1421	ACAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGG
1425	CUCUACC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGU
1429	CAGGGGC	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGA
1444	UCCUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUUC
1455	UCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCC
1482	GGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AACAACU
1484	CAUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAACAG
1493	GUUCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCACAG
1500	GGACCAU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCAU
1503	GAUUGAU	CUGAUGAGGCCGAAAGGCCGAA	AUAGUCC
1506	CGGUUAU	CUGAUGAGGCCGAAAGGCCGAA	AACAUAA

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1509	ACACGGU	CUGAUGAGGCGCGAAAGGCCGAA	AUGGUAG
1518	CGCCUGG	CUGAUGAGGCGCGAAAGGCCGAA	ACCAUGA
1530	CCAGAAU	CUGAUGAGGCGCGAAAGGCCGAA	AUUAUAG
1533	GGCCAC	CUGAUGAGGCGCGAAAGGCCGAA	AUGACCA
1551	AGCUGCU	CUGAUGAGGCGCGAAAGGCCGAA	AGGCCADG
1559	AGGUGGG	CUGAUGAGGCGCGAAAGGCCGAA	AGGUGCU
1563	GGUUAUA	CUGAUGAGGCGCGAAAGGCCGAA	ACAUAAAG
1565	GCGGUUA	CUGAUGAGGCGCGAAAGGCCGAA	AAACAUA
1567	UGGCGGU	CUGAUGAGGCGCGAAAGGCCGAA	AUAACA
1584	AUAUCCU	CUGAUGAGGCGCGAAAGGCCGAA	AUCUUC
1592	UAACUUG	CUGAUGAGGCGCGAAAGGCCGAA	AUAUCCU
1599	CCUUCUG	CUGAUGAGGCGCGAAAGGCCGAA	AACUUGU
1651	GCUCAGG	CUGAUGAGGCGCGAAAGGCCGAA	AGGUGGG
1661	CRAAGGA	CUGAUGAGGCGCGAAAGGCCGAA	AGGUUUC
1663	UUCAAAG	CUGAUGAGGCGCGAAAGGCCGAA	AAAGGUU
1678	CCAGGCU	CUGAUGAGGCGCGAAAGGCCGAA	AGGUCCU
1680	CCAGAGG	CUGAUGAGGCGCGAAAGGCCGAA	AGUGGCU
1681	GCCAGAG	CUGAUGAGGCGCGAAAGGCCGAA	AAGUGGC
1684	ACAGCCA	CUGAUGAGGCGCGAAAGGCCGAA	AGGAAGU
1690	AGAUGGA	CUGAUGAGGCGCGAAAGGCCGAA	AGUCCGG
1691	AAGAUGG	CUGAUGAGGCGCGAAAGGCCGAA	AAGUCCG
1696	CCACCCC	CUGAUGAGGCGCGAAAGGCCGAA	AUGGGCA
1698	CUCCAGG	CUGAUGAGGCGCGAAAGGCCGAA	AUAUCCG
1737	GCUGGUA	CUGAUGAGGCGCGAAAGGCCGAA	AGGUUUC
1750	UGAGGUG	CUGAUGAGGCGCGAAAGGCCGAA	AGCCGCC
1756	GGGCAGG	CUGAUGAGGCGCGAAAGGCCGAA	AGGCUUC
1787	UGGGGAC	CUGAUGAGGCGCGAAAGGCCGAA	AUGUCUC
1790	AUAAGAG	CUGAUGAGGCGCGAAAGGCCGAA	ACAADGC
1793	UCCAGCC	CUGAUGAGGCGCGAAAGGCCGAA	AGGACCA
1797	UUUAUGU	CUGAUGAGGCGCGAAAGGCCGAA	ACUGGUG
1802	UCUCCAG	CUGAUGAGGCGCGAAAGGCCGAA	AUCUGGU
1812	GGCCUGA	CUGAUGAGGCGCGAAAGGCCGAA	AUCCAGU
1813	UGAGGGU	CUGAUGAGGCGCGAAAGGCCGAA	AAUGCUG
1825	GCAGAGG	CUGAUGAGGCGCGAAAGGCCGAA	AGGUGGG
1837	GGAGCUA	CUGAUGAGGCGCGAAAGGCCGAA	AGGCCADG
1845	GGUGGOC	CUGAUGAGGCGCGAAAGGCCGAA	AGGCUUG
1856	AAGAUGG	CUGAUGAGGCGCGAAAGGCCGAA	AAGUCCG
1861	UACUGGA	CUGAUGAGGCGCGAAAGGCCGAA	AUCAUGU
1865	CUGAGGC	CUGAUGAGGCGCGAAAGGCCGAA	ACAAGUG
1868	UUUAUGU	CUGAUGAGGCGCGAAAGGCCGAA	ACUGGUG
1877	AGCUGCU	CUGAUGAGGCGCGAAAGGCCGAA	AGGCCADG
1901	GUCCCUU	CUGAUGAGGCGCGAAAGGCCGAA	AGUUUUA
1912	ACUGAUC	CUGAUGAGGCGCGAAAGGCCGAA	ACUAUAD
1922	UAACUUA	CUGAUGAGGCGCGAAAGGCCGAA	ACADUCA
1923	GAUACCU	CUGAUGAGGCGCGAAAGGCCGAA	AGCADCA
1928	CUGGUAA	CUGAUGAGGCGCGAAAGGCCGAA	ACUCUAA
1930	AGCUGGU	CUGAUGAGGCGCGAAAGGCCGAA	AAACUCU
1964	UGGGGAC	CUGAUGAGGCGCGAAAGGCCGAA	AUGUCUC
1983	UAACUUG	CUGAUGAGGCGCGAAAGGCCGAA	AUAUCCU

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1996	GGCUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCU
2005	GGUCCGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCA
2013	UACUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAADAGC
2015	CCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
2020	CUCAGAA	CUGAUGAGGCCGAAAGGCCGAA	AACCACC
2039	CCUCUGC	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGC
2040	CCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
2057	GGAUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCA
2061	ACAOGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAG
2071	CUGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGUG
2076	UAGCUCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCUAC
2097	CAUCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
2098	CGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUG
2115	AUCUCC	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGC
2128	CUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCUG
2130	GAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
2145	CAUCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
2152	AACUCUA	CUGAUGAGGCCGAAAGGCCGAA	AUUAUA
2156	UAUAUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAA
2158	AUUAUA	CUGAUGAGGCCGAAAGGCCGAA	AUACAUC
2159	AAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2160	AAAUUA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2162	CUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAUA
2163	AAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2166	AAUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAGU
2167	AAUUAU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2170	CUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AUAAUA
2171	GGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AACAACU
2173	CUGGUA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUA
2174	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUA
2175	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAACUCU
2176	UAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAAACUC
2183	CAUAUA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGU
2185	CUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCUG
2186	ACUCAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGCU
2187	UACUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGC
2189	GGUACUC	CUGAUGAGGCCGAAAGGCCGAA	AUAAUA
2196	CAUCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
2198	AACAUAA	CUGAUGAGGCCGAAAGGCCGAA	AGGCUCC
2199	AUAAACA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGGC
2200	CUUGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGA
2201	GCCGACA	CUGAUGAGGCCGAAAGGCCGAA	AAAACUU
2205	UCAGGOC	CUGAUGAGGCCGAAAGGCCGAA	ACAUAUA
2210	AGOCACU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCC
2220	AGAGAAC	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
2224	GGAUUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUAG
2226	GCGGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUCCA
2233	CCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
2242	GGUCCGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCA

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2248	UGGGADG	CUGAUGAGGCCGAAAGGCCGAA	AUGGADA
2254	UCAGUGU	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGA
2259	CACCGUG	CUGAUGAGGCCGAAAGGCCGAA	ADGUGAU
2260	GCAACGU	CUGAUGAGGCCGAAAGGCCGAA	AAUGUGA
2266	UCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCC
2274	UCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGU
2279	CUUGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCCUUC
2282	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
2288	AGGCCAU	CUGAUGAGGCCGAAAGGCCGAA	ACUUAUA
2291	AGCAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACCACUG
2321	CCCADGU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUC
2338	CAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCA
2339	CAAAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUUC
2341	AGGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUC
2344	GCUGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAAA
2358	CUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
2359	UCUGUUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGCAG
2360	UUCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGUU
2376	UCAGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACCACCU
2377	CUCAGAA	CUGAUGAGGCCGAAAGGCCGAA	AACCACC
2378	CAGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAACCCU
2379	CUUADGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGCA
2380	GCCGACA	CUGAUGAGGCCGAAAGGCCGAA	AAAACUU
2382	GGGGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAU
2384	UUGUGUC	CUGAUGAGGCCGAAAGGCCGAA	ACUGGAU
2399	GUCCACA	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUU
2401	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
2411	GCAUCCU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGUA
2417	ACGU AUG	CUGAUGAGGCCGAAAGGCCGAA	ACCADUC
2418	GGCCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCAGU
2425	AAOCCUC	CUGAUGAGGCCGAAAGGCCGAA	AOCCADG
2426	AAACUCU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUU
2433	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUA
2434	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAACUCU
2448	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
2449	GGGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUU
2451	AGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGGC
2452	GAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
2455	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
2459	GGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGUGG
2460	CGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUG
2479	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUC
2480	GGAU CAC	CUGAUGAGGCCGAAAGGCCGAA	AOGGUGA
2483	GGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGG
2484	GACUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAAAAAG
2492	AGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGCU
2504	ACAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGG
2508	UGGGADG	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUA
2509	CUGGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUAA

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2510	GCUGGUA	CUGAUGAGGCGGAAAGGCGGAA	AACUCUA
2520	CAUUGGG	CUGAUGAGGCGGAAAGGCGGAA	ACAAAAG
2521	UGAGGGU	CUGAUGAGGCGGAAAGGCGGAA	AADGCUG
2533	GAUACCU	CUGAUGAGGCGGAAAGGCGGAA	AGCAUCA
2540	CACAGCG	CUGAUGAGGCGGAAAGGCGGAA	ACUGCUG
2545	AGGACCA	CUGAUGAGGCGGAAAGGCGGAA	ACAGCAC
2568	UUUGACA	CUGAUGAGGCGGAAAGGCGGAA	ACUUCAC
2579	CAGGCGA	CUGAUGAGGCGGAAAGGCGGAA	AACUUAU
2585	AGAGAAC	CUGAUGAGGCGGAAAGGCGGAA	AUGCCAG
2588	AUUAGAG	CUGAUGAGGCGGAAAGGCGGAA	ACAAUGC
2591	AGGAGCA	CUGAUGAGGCGGAAAGGCGGAA	AGAACCA
2593	GCAGAGC	CUGAUGAGGCGGAAAGGCGGAA	AAAGAAG
2596	CAUUGGG	CUGAUGAGGCGGAAAGGCGGAA	ACAAAAG
2601	AAACGAA	CUGAUGAGGCGGAAAGGCGGAA	ACACGGU
2602	GGGAUGG	CUGAUGAGGCGGAAAGGCGGAA	AGCUGGA
2607	CCAGGUA	CUGAUGAGGCGGAAAGGCGGAA	AUCCGAG
2608	CACAGCG	CUGAUGAGGCGGAAAGGCGGAA	ACUGCUG
2609	UCCUGGU	CUGAUGAGGCGGAAAGGCGGAA	ACAUUCC
2620	GCAGGGU	CUGAUGAGGCGGAAAGGCGGAA	AGGUCCU
2626	GCTGGAA	CUGAUGAGGCGGAAAGGCGGAA	AUCGAAA
2628	AGGCUAC	CUGAUGAGGCGGAAAGGCGGAA	AGUGUGC
2635	AGGACCG	CUGAUGAGGCGGAAAGGCGGAA	AGCUGAA
2640	GGCAGGA	CUGAUGAGGCGGAAAGGCGGAA	ACAGGCC
2641	CUGCUGA	CUGAUGAGGCGGAAAGGCGGAA	AGCUGGG
2642	GAGGCAG	CUGAUGAGGCGGAAAGGCGGAA	AAACAGG
2653	GCAUCCU	CUGAUGAGGCGGAAAGGCGGAA	ACCAGUA
2659	CUUGCAC	CUGAUGAGGCGGAAAGGCGGAA	ACCCUUC
2689	CCUCCGA	CUGAUGAGGCGGAAAGGCGGAA	ACAUUAG
2691	GGCCUUG	CUGAUGAGGCGGAAAGGCGGAA	AGACAUU
2700	GGGCAGG	CUGAUGAGGCGGAAAGGCGGAA	AGGCUUC
2704	AGGCUGG	CUGAUGAGGCGGAAAGGCGGAA	AGAGGUC
2711	CUGCUGA	CUGAUGAGGCGGAAAGGCGGAA	AGCUGGG
2712	CCCUUCC	CUGAUGAGGCGGAAAGGCGGAA	AGAOCUC
2721	CUUGCAC	CUGAUGAGGCGGAAAGGCGGAA	ACCCUUC
2724	GCACAGG	CUGAUGAGGCGGAAAGGCGGAA	AUGUACC
2744	CUGCAGG	CUGAUGAGGCGGAAAGGCGGAA	ACCCACC
2750	GGUACUC	CUGAUGAGGCGGAAAGGCGGAA	AUAAAUU
2759	AGAUCGA	CUGAUGAGGCGGAAAGGCGGAA	AGUCCGG
2761	GCAGGGU	CUGAUGAGGCGGAAAGGCGGAA	AGGUCCU
2765	AGCGGCA	CUGAUGAGGCGGAAAGGCGGAA	AGCAAAA
2769	CCUGUUU	CUGAUGAGGCGGAAAGGCGGAA	ACAGACU
2797	GCACCAU	CUGAUGAGGCGGAAAGGCGGAA	AUUUCAU
2803	CGCCUGG	CUGAUGAGGCGGAAAGGCGGAA	ACCAUGA
2804	CUGCAGG	CUGAUGAGGCGGAAAGGCGGAA	ACCCACC
2813	GGGUCAG	CUGAUGAGGCGGAAAGGCGGAA	ACCGGAG
2815	AAAGUUG	CUGAUGAGGCGGAAAGGCGGAA	AGACUGU
2821	CCUCCAG	CUGAUGAGGCGGAAAGGCGGAA	AGGUCAG
2822	AAGUCCG	CUGAUGAGGCGGAAAGGCGGAA	AGGCTCC
2823	UGGGAGC	CUGAUGAGGCGGAAAGGCGGAA	AAAGGCA

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2829	AUGAUUA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAG
2837	UCAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AOCACCU
2840	CAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCA
2847	GGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGG
2853	AACAUAA	CUGAUGAGGCCGAAAGGCCGAA	AGGCUGC
2860	UCACAGU	CUGAUGAGGCCGAAAGGCCGAA	ACUUGGC
2872	CUUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCC
2877	GUGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCGGAA
2899	AAGAUCG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCG
2900	AAAACUC	CUGAUGAGGCCGAAAGGCCGAA	AAAUUAA
2904	AAUAGAG	CUGAUGAGGCCGAAAGGCCGAA	ADGAGU
2905	CAAUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAG
2906	UAAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAA
2907	AAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2908	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUC
2909	AGAGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCU
2910	AAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2911	AAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2912	GACAUUA	CUGAUGAGGCCGAAAGGCCGAA	AGAACAA
2913	UGACCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAAA
2914	CUUAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGCA
2915	UCUAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
2916	CUCCGGA	CUGAUGAGGCCGAAAGGCCGAA	ACGAUUA
2917	UCUCCGG	CUGAUGAGGCCGAAAGGCCGAA	AACGAUU
2918	CUCUCCG	CUGAUGAGGCCGAAAGGCCGAA	AAACGAA
2919	CGAOCUU	CUGAUGAGGCCGAAAGGCCGAA	ADGAGAA
2931	CUUCCGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
2933	CCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACCTC
2941	UGGGGAC	CUGAUGAGGCCGAAAGGCCGAA	ADGUUUC
2951	GCAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGCGUGG
2952	CACAGCG	CUGAUGAGGCCGAAAGGCCGAA	ACUGCUG
2955	UGACACA	CUGAUGAGGCCGAAAGGCCGAA	AGUCACU
2956	UUGAUUC	CUGAUGAGGCCGAAAGGCCGAA	AAGGAAA
2961	AGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACACAGA
2962	AAUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2965	CUUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCAAA
2966	CCUCUGC	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGC
2969	AAAACUU	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUU
2975	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUA
2976	AGUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AACCCUC
2977	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
2979	GGCAUUA	CUGAUGAGGCCGAAAGGCCGAA	AGAAUGA

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Table 6
Human ICAM Hairpin Ribozyme/Substrate Sequences

nt.		Hairpin Ribozyme Sequence		Substrate	
Position					
70	GGGCGGG	AGAA	GTUG	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
86	GGAGUGCG	AGAA	GCGC	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
343	CCCAUCAG	AGAA	GUUU	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
635	GGCTTUGG	AGAA	GCAG	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
653	UGUUCUCA	AGAA	GTUC	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
782	AGACUGGG	AGAA	GCCC	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
920	CTGCACAC	AGAA	GCCG	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
1301	ACAUUGGA	AGAA	GTUG	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
1373	CCCCGAUG	AGAA	GUGG	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
1521	AUGACUGC	AGAA	GTUA	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
1594	CTGUUGUA	AGAA	GUUU	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
2008	ACCCAAUA	AGAA	GCAA	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
2034	UTUCGUAA	AGAA	GUGG	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
2125	GGUCAGUA	AGAA	GCAG	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
2132	GGGUUGGG	AGAA	GUAG	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
2276	ACCTGUAC	AGAA	GUAC	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA
2810	AAGGUCAA	AGAA	GCAG	ACCAGAGAAACACAC	CGUUGUGGUACAUAUACCUUGUA

Table 7
Mouse ICAM Hairpin Ribozyme/Substrate Sequences
nt.
Position
Hairpin Ribozyme Sequence
Substrate

76	GGGAUCAC	AGAA	GUGA	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	UCACC	GUU	GUGAUCCC
164	UGAGGAAG	AGAA	GUUC	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	GAACU	GUU	CUUCCUCA
252	UCAGCTUCA	AGAA	GCUU	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	AAGCU	GUU	UGAGCTUGA
284	GCACAGCG	AGAA	GCTG	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	CAGCA	GUC	CGCUGUGC
318	AAGCGGAC	AGAA	GCAC	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	GUACA	GUC	GUCCGCTU
447	AGAGCTUG	AGAA	GCGG	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	CCGCG	GAC	CCAGCUCU
804	UCUCCUGG	AGAA	GCAU	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	AUGCC	GAC	CCAGGAGA
847	UCTAACCA	AGAA	GUGG	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	CCACU	GCC	UUGGUAQA
913	AGGAUCTG	AGAA	GCUA	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	UAGCG	GAC	CAGAUCCTU
946	AGUGUGUA	AGAA	GUUA	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	UAACA	GUC	UACAACU
1234	CCCAAGCA	AGAA	GUUC	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	AGACG	GAC	UGCTUUGG
1275	AUUCAGA	AGAA	GCTG	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	CAGCA	GAC	UTUGAAAU
1325	UGCCUCC	AGAA	GCAG	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	CUGCA	GAC	GGAGGCA
1350	CCCCGAG	AGAA	GCAG	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	CUGCU	GCC	CAUCGGGG
1534	ACAUAGA	AGAA	GCCA	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	UGGCA	GCC	UCUUAUGU
1851	GUCCACCG	AGAA	GUAG	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	CUACA	GCC	CGGUGGAC
1880	AGAAUGAA	AGAA	GCGU	ACCAGAGAAAACACACGUGUGGUA	CAUUUACCTUGGUA	ACGCU	GAC	UUCAUUCU

Table 8			Rat ICAM Hairpin Ribozyme/Substrate Sequences	
nt.			Hairpin Ribozyme Sequence	
Position		Substrate		
5	AAAGUGCA AGAA GCAG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	CUGCU GCC UGCACUUA		
59	GGAGCCAGA AGAA GCAU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	AUGCU GCC UUGGCUCC		
84	GGGAUCAC AGAA GCGA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	UCGCC GUU GUGAUCCC		
295	GCACAGUG AGAA GCTUG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	CAGCA GAC CACUGUGC		
329	AAGCCGAG AGAA GCGU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	ACGCA GUC CUCGGCTU		
433	UUCACCCA AGAA GCGC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	GCGCU GCC UGGUGGAA		
626	CAUUCUUG AGAA GUGA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	UCACU GUU CAAGAUAU		
806	UTCUCAGG AGAA GCAU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	AUGCU GAC CCUGGAGA		
849	UCCACUGA AGAA GUGG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	CCACU GCC UCAGUGGA		
915	AGGUCUG AGAA GCCA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	UGGCG GAC CAGACCCU		
1182	ACUCCAA AGAA GCAG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	CUGCG GCC UUGGAGGU		
1307	AUGUAAGA AGAA GCTUG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	CAGCA GAC UCUUACAU		
1357	UGCUUUC AGAA GCGG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	CUGCA GCC GGAAGCA		
1382	UCCCGAUA AGAA GCGG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	CCGCU GCC UAUCGGGA		
1858	GCCCACCA AGAA GUAG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	CUACA GCC UGGUGGCG		
1887	AGAAAGAA AGAA GCTU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	AGGCU GAC UUCCTUCU		
2012	GAGUUGG AGAA GUGU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	ACACU GUC CCCAACTC		
2303	AGACUCCA AGAA GUGG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	CCACA GCC UGGAGUCU		
2539	CCUCCAC AGAA GCUU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGGUA	AAGCU GUU GUGGAGG		

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Table 9: Rat ICAM HH Ribozyme Target Sequence

nt. Position	HH Target sequence	nt. Position	HH Target Sequence
11	GAUCCAAU U CACACUGA	394	GUGGUGCU U CUGAACAG
23	GCUGACUU C CUUCUCUA	420	GCACCCCU C CCAGGGCA
26	GAACUGCU C UUCUCUCU	425	CCUGGGCU U CUCOCACC
31	CCUCUGCU C CUGGUCCU	427	UCCUGUUU U AAAAACCA
34	CUGAAGCU C AGAUAUAC	450	AAGAACC U ADCCUGGG
40	CUCAAGGU A CAAGCCCC	451	GGGUACUU C CCCCAGGC
48	GAGAACCU C GGGCUGGG	456	CUUGGCUU C UGOCACCA
54	CCCCGCUU C CCUGAGCC	495	GCCACCAU C ACUGUGUA
58	CCUGGCUU U UAGCUCUC	510	GUGGUGCU C CGUGGGA
64	CAUUGGCU U CAACCCGU	564	GAAAADGU U CCAACCA
96	CCUCUGCU C CUGGUCUU	592	GGGAGUAD C ACCAGGGA
102	CUCCUGGU C CUGGUGGC	607	GAGCCAAU U UCUCAUCC
108	GGACUGCU U GGGGAACU	608	AGCCAAU U CUCAUGCU
115	UCCUACCU U UGUUCCCA	609	GCCAAUUU C UCADGCUU
119	GACACUGU C CCCCACUC	611	CAUUUUU C AUGCUUCA
120	GUUGUGAU C CCCCAGGC	656	GUCACUGU U CAAGAAUG
146	CCAGACCU U GGAACUCC	657	UCACUGUU C AAGAAUGU
152	ACCCGGCU C CACCUCAA	668	GAACUGCU C UUCUCUUU
158	AUUUCUUU C ACCAGUCA	677	GCACCCCU C CCAAGGCA
165	UGAACAGU A CUUCUUCC	684	AGGCAGCU C CGGACUUU
168	GAAGCCUU C CUGCCUUG	692	CCAGACCU U GGAACUCC
185	GGGUGGAU C CGUGCAGG	693	CGGACUUU C GADCUUCC
209	CAGCCCCU A AUCUGACC	696	GCCUGUUU C CCGCCUCC
227	GACCAAGU A ACUGUGAA	709	CAGCAUUU A CCCCUCAC
230	CAAGCUGU U GUGGGAGG	720	CUACAACU U UUCAGCUC
237	CUGAAGCU C GACACCCC	723	CAACUUUU C AGCUCCCA
248	GGCCCCCU A CCUUAGGA	735	CUCCUGGU C CUGGUUGC
253	CACUGCCU C AGUGGAGG	738	UCCUGCCU C GGGUGGA
263	GAGCCAAU U UCUCAUCC	765	ACUGUGCU U UGAGAACT
267	GAAGCCUU C CUGCCUUG	769	UCUUGUGU U CCCCUGAA
293	GAAGCUCU U CAAGCUGA	770	CUUGUGUU C CCUGGAAG
319	CGGAGGAU C ACAAACGA	785	AGGCCUGU U UCCUGCCU
335	ACUGUGCU U UGAGAACU	786	GGCCUGUU U CCUGCCUC
337	UGUGCUAU A UGGUCCUC	792	CUCCUGGU C CUGGUUGC
338	AAGCUCUU C AAGCUGAG	794	UCCUGCCU C UGAAGCUC
359	CACGCAGU C CUUGGCUU	807	GCUCAGAU A UACUGGA
367	CAUUGGCU U CAACCCGU	833	CCUGGGGU U GGAACUA
374	UAACCCCU C ACCACCUU	846	CUGACAGU U AUUUUUG
375	AGAAGCCU U CCUGCCUC	851	GCUCACCU U UAGCAGCU
378	ACCCACCU C ACAGGGUA	863	CAUUGGCU U CAACCCGU
386	CGCUGUGU U UUGGAGCU	866	CCAUCCUU C CUCGACA

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867	GACCACTU C CCCAGCTA	1421	GGGUAUUA C CCCAGGC
869	CUCUUCUU C UUGGGAAG	1425	ACCACTU C CUCUGGU
881	AADGGCUU C AACCGUG	1429	AUAUUGU A GGUUAGG
885	GACCAAGU A ACUGUGAA	1444	AGAAGGU C AGGAGGAG
933	UGUGUAUU C GUUCCAG	1455	GGGUAUUA C ACCAGGGA
936	GCAGAGAU U UUGUGUA	1482	AGGUAUUA U CCCAGG
978	UUGAGAAU C UACAACUU	1484	ACUGGUU U CUCUUGC
980	GAGAAUUA A CAACUUU	1493	CCUGGGU U GGAGACUA
986	CUACAACU U UACAGCUC	1500	CGUAAAU U AUGGUCA
987	UACAACUU U UACAGCUC	1503	GAAAUUGU U CCAACCAC
988	ACAACUUU U CAGCUCUC	1506	UGGUAUUA A AUUGUUGG
1005	UUGUGAU C GUGGUGUC	1509	GCAACAU C ACUGUGUA
1006	GUGGAGU A UACAGG	1518	GUGGUGU C GCGUUGU
1023	CCGAGGU C UACAGG	1530	ACUGGUU C AUAAUUGU
1025	GGAGGUU C AGAAGGG	1533	CUGUAUUA U GGGGCUU
1066	CCUACUUU U GUUCCAA	1551	GUGGCUU C UGUGUA
1092	AGAGGGU C UACAGGA	1559	UGGAGU C CUGUUA
1093	AGGGAAU C CAGCCCU	1563	UCCUACU U UGUUCCA
1125	CCCCAUA C UGUUGAU	1565	UUAACUU A UUAAGCC
1163	ACGAGCU U CUUUGCU	1567	ACACUAU U ACGGAG
1164	CGAGGUU C UUUUGCUC	1584	AGGAAGU C AGGAUAUA
1166	ACGUUCU U UUGCUCU	1592	CAGGAUA A CAAGUAC
1172	CUUUGCU C UGGGCUU	1599	UACAAGU A CAGAAGG
1200	AUCCAUU C ACACUGAA	1651	CCCGCUU C CUGAGCC
1201	UUGGCUU C UACAGG	1661	CUGACUU U GCGUGGU
1203	GGGCUUC C CACAGGUC	1663	GAACAGU C AAUGGACA
1227	UUGGAACU C CAUGUGU	1678	GAGAACU C GCGUGGG
1228	GCGGCUU C GUGAUUGU	1680	GGGCUUC C CACAGGUC
1233	CUCUGGU C CUGGUGC	1681	GCGUGUU U CUGGUC
1238	UGUGUAU A UGUUCCUC	1684	CUGGUGU A GACGUC
1264	GGAAGAU C AUACGGU	1690	CCCCAUA A CAUACAU
1267	GUCACUGU U CAAGAUG	1691	CCGACUU U CGAUUUC
1294	CAGAGAUU U UGUGUAG	1696	CUCUGGU C CUGGUGC
1295	AGAGGGU C UACAGGA	1698	UACAUUA A CCUGGAGA
1306	AGCAGAU C UUAACUC	1737	GAUACAU U CACGGUGC
1321	AACAGAU C UGGGAAA	1750	GUCAUUU A CACUAUU
1334	GUUUGUU U CCAAGAGC	1756	CCUGGUU C CUGGUCU
1344	UUGGUGU C AGGUUUC	1787	GAGAACU C GCGUGGG
1351	UCAGGCU A AGAGGACU	1790	GACACUGU C CCAACUC
1353	UAGCAGU C AACAAUGG	1793	AUGGUUU C ACCUGGAC
1366	AGGUAUUA U CCCCAGG	1797	UCCUGUU U AAAAACA
1367	GGGUAUUA C CCCAGGC	1802	GUCAGAU A UACUGGA
1368	GAUGGUGU C CCGUGGC	1812	AACAGAU C UGGGAAA
1380	CUGGUAU C GGGAGGU	1813	GCGGCUU C GUGAUUGU
1388	UGGAGAU A ACUGGAG	1825	GCAACAU C ACUGUGUA
1398	CUGGUGU C ACAGGACA	1837	ACCACTU C ACAGGUA
1402	CUGGCUU U GAGACUG	1845	AGAGGACU C GAGGGGC
1408	UUGUGAU C GUGGUGUC	1856	CCCCUAU C UGACUUC
1410	CGAACUAU C GAGUGGAC	1861	CAUGUGU A UAUUGUC

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1865	UADCCGGU	A	GACACAAG	2198	GAADGUCU	C	CGAGGUCA
1868	UCACGAGU	C	AUADAAAU	2199	AGACUCUU	A	CADGCCAG
1877	ACAGUACU	U	CCCCCAGG	2200	GGGUACUU	C	CCCCAGGC
1901	CUAAAACU	C	AAGGUACA	2201	GGGCUUCU	C	CACAGGUC
1912	GAACAGAU	C	AADGGACA	2205	UUUUGUGU	C	AGCCACTG
1922	ADGUAGAU	U	AUUGCCUA	2210	UGGAGACU	A	ACUGGADG
1923	UGGACGGU	C	ACCUUUAG	2220	GAGAACCU	C	GGCCUGGG
1928	GUUCAGAU	A	UACCCUGA	2224	ACAUACAU	U	CCUACCCU
1930	UGGAGACU	A	ACUGGADG	2226	CUGAACCU	C	AGGCCACA
1964	AGAGAUUU	U	GGGUCAGC	2233	UCADGCUU	C	ACAGAACU
1983	GAGAACCU	C	GGCCUGGG	2242	ACACAGCU	C	UCAGUAGU
1996	UGGAGACU	C	UUCAAGCU	2248	CUCCUGGU	C	CUGGUCGC
2005	ADGUAGAU	U	AUUGCCUA	2254	AUCCAAUU	C	ACACUGAA
2013	CGCUGCCU	A	UCGGGAUG	2259	GAUCACAU	U	CACGGUGC
2015	CUGCCUAU	C	GGGADGGU	2260	AUCACAUU	C	ACGGUGCU
2020	UADUGAGU	A	CCCUGUAC	2266	AUCAGGAU	A	UACAAGUU
2039	CGGAGGAU	C	ACAAACGA	2274	GAGCAGGU	U	AACAUGUA
2040	CCUGAACU	C	CUGGAGGU	2279	GGAAAGAU	C	AUAAGGGU
2057	CUGGUCCU	C	CAADGGCU	2282	ACAGUUUU	U	UADUGAGU
2061	GGGUCCAU	U	UACACCUA	2288	GGCCUGGU	C	CUCCAADG
2071	AUACUUGU	A	GGCUCAGG	2291	CAGGAUAU	A	CAAGUUAC
2076	UGUAGCCU	C	AGGCCUAA	2321	GGAAAGAU	C	AUAAGGGU
2097	CCACUCUU	U	GUUGAUGU	2338	UUGGGCUU	C	UCCACAGG
2098	CCUGACCU	C	CUGGAGGU	2339	GGGUACUU	C	CCCCAGGC
2115	UCCCGACU	A	GGGUCCUG	2341	GGGCUUGU	C	GGUGCUCA
2128	AGUGCCGU	A	CCAUAGUC	2344	CUGCUUGU	A	GAOCUCUC
2130	GGCUGUUU	C	CUGCCUCU	2358	CCCUGCCU	C	CUCCACCA
2145	CCACUCUU	U	GUUGAUGU	2359	CCAUCCAU	C	CCACAGAA
2152	UUGAGAAU	C	UACAACUU	2360	CUUGGCUU	C	CCUGGAAG
2156	UGACAGUU	A	UUUAUUGA	2376	GAACUGCU	C	UUCCUCUU
2158	UGAUGUAU	U	UADUAAUU	2377	GACUCCUU	U	CUUAUUA
2159	GAUGUAUU	U	AUUAUUUC	2378	GCUGAUUU	C	UUUACOGA
2160	ADGUAAUU	A	UUAAUCCA	2379	CUGCUCUU	C	CUUUUGOG
2162	ACAUUCCU	A	CCUUUGUU	2380	UGAUUUUU	U	UACAGAGU
2163	UAUUUAUU	A	AUUCAGAG	2382	AUUUCUUU	C	ACGAGUCA
2166	UGAUGUAU	U	UADUAAUU	2384	UADCCGGU	A	GACACAAG
2167	GAUGUAUU	U	AUUAUUUC	2399	UAAAUACU	A	UGGGGACG
2170	GUUUUAUU	U	AADUCAGA	2401	UGUGCUAU	A	UGGUCCUC
2171	CAGUAUUU	U	AUUGAGUA	2411	CAAUUUUU	C	AUGCUUCA
2173	UGUGCUAU	A	UGGUCCUC	2417	AUCAGGAU	A	UACAAGUU
2174	UCUCUAUU	A	CCCCUGCU	2418	UCAUGCUU	C	ACAGAACU
2175	AUUUCUUU	C	ACGAGUCA	2425	UUADUAAU	U	CAGAGUUC
2176	GAAAADGU	U	CCAAACCAC	2426	CCUGGGGU	U	GGAGACTA
2183	UGACAGUU	A	UUUAUUGA	2433	UCAGAGUU	C	UGACAGUU
2185	ACAGUUUU	U	UAUUGAGU	2434	CGGAGGAU	C	ACAAACGA
2186	CAGUUAUU	U	AUUGAGUA	2448	UGAACAGU	A	CUUUUUUU
2187	AGUUAUUU	A	UUGAGUAC	2449	GAAGCCUU	C	CUUGCCUG
2189	UUUUUAUU	U	GAGUACCC	2451	GGCCUGUU	U	CCUGCCUC
2196	CGACAGU	U	AUUUAUUG	2452	GCCUGUUU	C	CUGCCUCU

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2455	ACAUUCCU	A	CCUUUGUU	2761	CGGACUUU	C	GAUCUUCC
2459	CCUGGCUU	C	CUCCCAAC	2765	CUUUUGCU	C	UGGGGCUU
2460	CCUACCUU	U	GUUCCCAA	2769	UUCUCUAA	U	ACCCUUGC
2479	UUAACCUU	A	UUACCGGC	2797	CGUGAAAU	U	AUGGUCAA
2480	GUUGCGUU	U	GUGAUCCC	2803	CUCAUGCU	U	CACAGAAC
2483	ACCUUUGU	U	CCCAADGU	2804	UCAUGCUU	C	ACAGAAAU
2484	CCUUUGUU	C	CCADUGUC	2813	GUUCCCAU	C	CUGACCCU
2492	GACCAUCC	C	CCCAUCCA	2815	CGGACUUU	C	GAUCUUCC
2504	ACCUACAU	A	CAUUCUUA	2821	CCUGACCU	C	CUGGAGGU
2508	ACAUACAU	U	CCUACCUU	2822	UACUACUU	U	UCAGCUCC
2509	CAUACAUU	C	CUACCUUU	2823	CAACUUUU	C	AGCUCCCA
2510	GUCCAUUU	A	CAUUAUUU	2829	UUGGUGCU	C	AGGUADCC
2520	ACCUUUGU	U	CCCAADGU	2837	CACAGGGU	A	CUUCCCCC
2521	CCUUUGUU	C	CCADUGUC	2840	GCACCCCU	C	CCAGCGCA
2533	ACAGCAUU	U	ACCCCUCA	2847	UUAUCCCU	C	ACCCACCU
2540	UUGGUGCU	C	AGGUADCC	2853	UUCGACUU	U	CCGACUAG
2545	AGGACGCU	C	CGGACUUU	2860	UCUUGUGU	U	CCCUGGAA
2568	CAGAGAUU	U	UGUGUCAG	2872	GGGCUUGU	C	GGGCUCCA
2579	CCUGCAUU	U	UGCCUUGG	2877	UGGAGUCU	C	CCAGCACC
2585	CUUCUUGU	A	GAUUCUUC	2899	AGGACGCU	C	CGGACUUU
2588	UGCCUCCU	C	CCACAGCC	2900	GGCUGACU	U	CCUUCUCU
2591	CUUUCUCC	C	UUGCGAAG	2904	GAACUGCU	C	UUUCUCUU
2593	UCUCUAAU	A	CCCUUGCU	2905	GGCUGACU	U	CCUUCUCU
2596	CUUCCUGU	C	CUUGUUGC	2906	GUUGAUUU	A	UUUAUUAA
2601	UGUGCUAU	A	UGGUCCUC	2907	CGGCUCCU	C	CUUUGCGG
2602	GUUCCUGU	C	GUUUGUGU	2908	UGAUUUUU	U	UAUUAAUU
2607	GUGGGAGU	A	UCACCAAG	2909	GAACUGCU	C	UUUCUCUU
2608	CUUUAAGU	C	CCGUGGGA	2910	ACUUCUCC	C	UCUUAUAC
2609	UGGAGACU	A	ACUGGAUG	2911	UUCCUUCU	C	UAUUACCC
2620	UCAGAGUU	C	UGACAGUU	2912	AUGUAUUU	A	UUAAUCCA
2626	CUUCACAU	A	GUGCUGCU	2913	UGUGUAUU	C	GUUCCACG
2628	UACAACUU	U	UCAGCUCC	2914	GUUUUUUU	U	AAUUUAGA
2635	UCACAGAU	C	CAAUUCAC	2915	UAUUUUUU	A	AUUUAGAG
2640	GUUCAGGU	A	UCCAUCCA	2916	CUUUCUCC	C	UUGCGAAG
2641	CCCCACCU	A	CAUACAUU	2917	CUUUCUCC	U	GCGAAGAC
2642	GUUUGUUU	C	CUUCCUCC	2918	AUUUCUUU	C	ACGAGUCA
2653	CCACAGGU	C	AGGGUGCU	2919	UUUUGUGU	C	AGCCACUG
2659	AGAAGGGU	C	CUGCAAGC	2931	GAUGGUGU	C	CGGCUGOC
2689	ACUAGGGU	C	CUGAAGCU	2933	UGGAGUCU	C	CCAGCACC
2691	UCAGGCUU	A	AGAGGACU	2941	CAGUACUU	C	CCCCAGGC
2700	AGGGUACU	U	CCCCAGAG	2951	ACCADGCU	U	CCUUCGAC
2704	GACCAUCC	C	CCCAUCCA	2952	CGGACUUU	U	CGAUUUUC
2711	CCCUACCU	U	AGGAAGGU	2955	UGCUUCCU	C	UGACADGG
2712	CCUACCUU	A	GGAAGGUG	2956	CUUUCUCC	U	GAUUCAAU
2721	GGAAAGAU	C	AUACGGGU	2961	UUUUGUGU	C	AGCCACUG
2724	AAGAUCAU	A	CGGCUUUG	2962	UGUGUAUU	C	GUUCCACG
2744	GGGUGGAU	C	CGUGCAGG	2965	CUUUGAAU	C	AAUUAAGU
2750	GUUCCUGU	U	UAAAAACC	2966	UGGAAGCU	C	UUCAAGCU
2759	GACGAACU	A	UCGAGUGG	2969	GAUUCAAU	A	AAGUUUUA

2975 UGGAAGCU C UUCAAGCU
2976 UAUUAGGU C CUCACCUG
2977 GAAGCUCU U CAAGCUGA

Table 10: Rat ICAM HH Ribozyme Sequences

nt: Position	Rat HH Ribozyme Sequence
11	UCAGUGUG CUGAUGAGGCGCGAAAGGCGCGAA AUUGGADC
23	UAGAGAAG CUGAUGAGGCGCGAAAGGCGCGAA AAGUCAGC
26	AAGAGGAA CUGAUGAGGCGCGAAAGGCGCGAA AGCAGUUC
31	AGGACCAG CUGAUGAGGCGCGAAAGGCGCGAA AGCAGAGG
34	GUADAUCU CUGAUGAGGCGCGAAAGGCGCGAA AGCUUCAG
40	GGGSCUUG CUGAUGAGGCGCGAAAGGCGCGAA ACCUUGAG
48	CCCAGGCC CUGAUGAGGCGCGAAAGGCGCGAA AGGUUCUC
54	GGCUCAGG CUGAUGAGGCGCGAAAGGCGCGAA AGGCGGGG
58	GGGAGCUA CUGAUGAGGCGCGAAAGGCGCGAA AGGCAAGG
64	AAGGGUUG CUGAUGAGGCGCGAAAGGCGCGAA AGCCAUUG
96	AGGACCAG CUGAUGAGGCGCGAAAGGCGCGAA AGCAGAGG
102	GCGACCAG CUGAUGAGGCGCGAAAGGCGCGAA ACCAGGAG
108	AGUCCCC CUGAUGAGGCGCGAAAGGCGCGAA AGCAGUCC
115	UGGGAACA CUGAUGAGGCGCGAAAGGCGCGAA AGGUAGGA
119	GAGUUGGG CUGAUGAGGCGCGAAAGGCGCGAA ACAGUGUC
120	GGCCCGGG CUGAUGAGGCGCGAAAGGCGCGAA AUCAACAAC
146	GGAGUUC CUGAUGAGGCGCGAAAGGCGCGAA AGGUUCUG
152	UUGAGGUG CUGAUGAGGCGCGAAAGGCGCGAA AGCCGGGU
158	UGACUUGU CUGAUGAGGCGCGAAAGGCGCGAA AAAGAUAU
165	GGGGGAAG CUGAUGAGGCGCGAAAGGCGCGAA ACUGUACA
168	CGAGGCAG CUGAUGAGGCGCGAAAGGCGCGAA AAGGCUUC
185	CCUGCAAG CUGAUGAGGCGCGAAAGGCGCGAA AUCCACCC
209	GGUCAGAU CUGAUGAGGCGCGAAAGGCGCGAA AGGGGCUG
227	UUCACAGU CUGAUGAGGCGCGAAAGGCGCGAA ACUUGGUC
230	CCUCCAC CUGAUGAGGCGCGAAAGGCGCGAA ACAGCUUG
237	GGGGUGUC CUGAUGAGGCGCGAAAGGCGCGAA AGCUUCAG
248	UCCUAAGG CUGAUGAGGCGCGAAAGGCGCGAA AGGGGGCC
253	CCUCCACT CUGAUGAGGCGCGAAAGGCGCGAA AGGCAGUG
263	GCAUGAGA CUGAUGAGGCGCGAAAGGCGCGAA AUUGGCUUC
267	CGAGGCAG CUGAUGAGGCGCGAAAGGCGCGAA AAGGCUUC
293	UCAGCUUG CUGAUGAGGCGCGAAAGGCGCGAA AGAGCUUC
319	UUGUUUGU CUGAUGAGGCGCGAAAGGCGCGAA AUCCUCCG
335	AGUUCUCA CUGAUGAGGCGCGAAAGGCGCGAA AGCACAGU
337	GAGGACCA CUGAUGAGGCGCGAAAGGCGCGAA AUAGCACA
338	CUCAGCUU CUGAUGAGGCGCGAAAGGCGCGAA AAGAGCUU
359	AAGCCGAG CUGAUGAGGCGCGAAAGGCGCGAA ACUGGUGU
367	ACGGGUUG CUGAUGAGGCGCGAAAGGCGCGAA AGCCAUUG
374	AGGUGGGU CUGAUGAGGCGCGAAAGGCGCGAA AGGGGUAA
375	GAGGCAGG CUGAUGAGGCGCGAAAGGCGCGAA AGGCUUCU
378	UACCCUGU CUGAUGAGGCGCGAAAGGCGCGAA AGGUGGGU
386	AGCUCCAA CUGAUGAGGCGCGAAAGGCGCGAA ACACAGCG

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394	CUGUUCAG	CUGAUGAGGCOGAAAGGCOGAA	AGCACCAC
420	UGGCGUGG	CUGAUGAGGCOGAAAGGCOGAA	AGGGGUGC
425	GGUGGCAG	CUGAUGAGGCOGAAAGGCOGAA	AGCOGAGG
427	UGGUUUUU	CUGAUGAGGCOGAAAGGCOGAA	AACAGGGA
450	CGCAGGAU	CUGAUGAGGCOGAAAGGCOGAA	AGGUUCUU
451	GCCUGGGG	CUGAUGAGGCOGAAAGGCOGAA	AAGUACCC
456	UGGUGGCA	CUGAUGAGGCOGAAAGGCOGAA	AAGCOGAG
495	UACACAGU	CUGAUGAGGCOGAAAGGCOGAA	AUGGUGGC
510	UUCOCACG	CUGAUGAGGCOGAAAGGCOGAA	AGCAGCAC
564	GUGGUUGG	CUGAUGAGGCOGAAAGGCOGAA	ACAUUUUC
592	UCCCGUGU	CUGAUGAGGCOGAAAGGCOGAA	AUAUCCOC
607	GCAUGAGA	CUGAUGAGGCOGAAAGGCOGAA	AUUGGCUU
608	AGCAUGAG	CUGAUGAGGCOGAAAGGCOGAA	AAUUGCUU
609	AAGCAUGA	CUGAUGAGGCOGAAAGGCOGAA	AAAUUGGC
611	UGAAGCAU	CUGAUGAGGCOGAAAGGCOGAA	AGAAAUUG
656	CAUUCUUG	CUGAUGAGGCOGAAAGGCOGAA	ACAGUGAC
657	ACAUUCUU	CUGAUGAGGCOGAAAGGCOGAA	AACAGUGA
668	AAGAGGAA	CUGAUGAGGCOGAAAGGCOGAA	AGCAGUUC
677	UGGCGUGG	CUGAUGAGGCOGAAAGGCOGAA	AGGGGUGC
684	AAAGUCCG	CUGAUGAGGCOGAAAGGCOGAA	AGCUUCUU
692	GGAGUUCU	CUGAUGAGGCOGAAAGGCOGAA	AGGUUCUG
693	GGAGAUUC	CUGAUGAGGCOGAAAGGCOGAA	AAAGUCCG
696	AGAGGCAG	CUGAUGAGGCOGAAAGGCOGAA	AAACAGGC
709	GUGAGGGG	CUGAUGAGGCOGAAAGGCOGAA	AAAUUCUU
720	GAGCUGAA	CUGAUGAGGCOGAAAGGCOGAA	AGUUGUAG
723	UGGGAGCU	CUGAUGAGGCOGAAAGGCOGAA	AAAAGUUG
735	GCGACCAG	CUGAUGAGGCOGAAAGGCOGAA	ACCAGGAG
738	UCCACCCC	CUGAUGAGGCOGAAAGGCOGAA	AGGCAGGA
765	AGUUCUCA	CUGAUGAGGCOGAAAGGCOGAA	AGCACAGU
769	UUCACAGG	CUGAUGAGGCOGAAAGGCOGAA	ACACAAGA
770	CUUCACAG	CUGAUGAGGCOGAAAGGCOGAA	AACACAAG
785	AGGCAGGA	CUGAUGAGGCOGAAAGGCOGAA	ACAGGCUU
786	GAGGCAGG	CUGAUGAGGCOGAAAGGCOGAA	AACAGGCC
792	GCGACCAG	CUGAUGAGGCOGAAAGGCOGAA	ACCAGGAG
794	GAGCUUCA	CUGAUGAGGCOGAAAGGCOGAA	AGGCAGGA
807	UCCAGGUA	CUGAUGAGGCOGAAAGGCOGAA	AUCUGAGC
833	UAGUCUCC	CUGAUGAGGCOGAAAGGCOGAA	ACCCCAGG
846	CAAUAAAU	CUGAUGAGGCOGAAAGGCOGAA	ACUGUCAG
851	AGCUGCUA	CUGAUGAGGCOGAAAGGCOGAA	AGGUGAGC
863	ACGGGUUG	CUGAUGAGGCOGAAAGGCOGAA	AGCCAUUG
866	UGOCAGAG	CUGAUGAGGCOGAAAGGCOGAA	AAGCAUGG
867	UAGGUGGG	CUGAUGAGGCOGAAAGGCOGAA	AGGUGGUC
869	CUUCGCAA	CUGAUGAGGCOGAAAGGCOGAA	AGGAAGAG
881	CACGGGUU	CUGAUGAGGCOGAAAGGCOGAA	AAGCCAUU
885	UUCACAGU	CUGAUGAGGCOGAAAGGCOGAA	ACUUGGUC
933	CUGGGAAC	CUGAUGAGGCOGAAAGGCOGAA	AAUACACA
936	UGACACAA	CUGAUGAGGCOGAAAGGCOGAA	AUCUCUGC
978	AAGUUGUA	CUGAUGAGGCOGAAAGGCOGAA	AUUCUCAA
980	AAAAGUUG	CUGAUGAGGCOGAAAGGCOGAA	AGAUUCUC

986	GAGCUGAA	CUGAUGAGGCOGAAAGGCOGAA	AGUUGUAG
987	GGAGCUGA	CUGAUGAGGCOGAAAGGCOGAA	AAGUUGUA
988	GGGAGCUG	CUGAUGAGGCOGAAAGGCOGAA	AAAGUUGU
1005	GACGCCAC	CUGAUGAGGCOGAAAGGCOGAA	AUCACGAA
1006	CCUGGUGA	CUGAUGAGGCOGAAAGGCOGAA	ACUCCAC
1023	CCUUCUGA	CUGAUGAGGCOGAAAGGCOGAA	ACCUCGG
1025	CCCCUUCU	CUGAUGAGGCOGAAAGGCOGAA	AGACUCC
1066	UUGGEAAC	CUGAUGAGGCOGAAAGGCOGAA	AAGGUAGG
1092	UCUGCUGA	CUGAUGAGGCOGAAAGGCOGAA	ACCCUUCU
1093	AGGGGCUG	CUGAUGAGGCOGAAAGGCOGAA	AGUCCUUC
1125	ADCAACAA	CUGAUGAGGCOGAAAGGCOGAA	AGUUGGG
1163	AGCAAAAG	CUGAUGAGGCOGAAAGGCOGAA	AGGUUGU
1164	GAGCAAAA	CUGAUGAGGCOGAAAGGCOGAA	AAGGUUG
1166	CAGAGCAA	CUGAUGAGGCOGAAAGGCOGAA	AGAAGGU
1172	AGGCCGCA	CUGAUGAGGCOGAAAGGCOGAA	AGCAAAAG
1200	UUCAGUGU	CUGAUGAGGCOGAAAGGCOGAA	AADUGGAT
1201	CCUGUGGA	CUGAUGAGGCOGAAAGGCOGAA	AAGGCCAA
1203	GACCUUGG	CUGAUGAGGCOGAAAGGCOGAA	AGAAGGCC
1227	AGCAC AUG	CUGAUGAGGCOGAAAGGCOGAA	AGUUCCAA
1228	ACGAUCAC	CUGAUGAGGCOGAAAGGCOGAA	AAGGCCGC
1233	GCGAC CAG	CUGAUGAGGCOGAAAGGCOGAA	ACCAGGAG
1238	GAGGACCA	CUGAUGAGGCOGAAAGGCOGAA	AUAGCACA
1264	ACCCGUAT	CUGAUGAGGCOGAAAGGCOGAA	AUCUUUCC
1267	CADUCUUG	CUGAUGAGGCOGAAAGGCOGAA	ACAGUGAC
1294	CUGACACA	CUGAUGAGGCOGAAAGGCOGAA	AADUCUG
1295	UCUGCUGA	CUGAUGAGGCOGAAAGGCOGAA	ACCCUUCU
1306	GCAUGUAA	CUGAUGAGGCOGAAAGGCOGAA	AGUCUGCU
1321	UUUCCCA	CUGAUGAGGCOGAAAGGCOGAA	ACUCUGUU
1334	GCUCUGGG	CUGAUGAGGCOGAAAGGCOGAA	ACGABUAC
1344	GGAIACCU	CUGAUGAGGCOGAAAGGCOGAA	AGCAACGA
1351	AGUCCUUC	CUGAUGAGGCOGAAAGGCOGAA	AGGCCUGA
1353	CCAUUGUU	CUGAUGAGGCOGAAAGGCOGAA	AGCUGCUA
1366	CCUGGGGG	CUGAUGAGGCOGAAAGGCOGAA	AGUACCCU
1367	GCCUGGGG	CUGAUGAGGCOGAAAGGCOGAA	AAGUACCC
1368	GGCAGCGG	CUGAUGAGGCOGAAAGGCOGAA	ACACCAUC
1380	ACCAUCCC	CUGAUGAGGCOGAAAGGCOGAA	AUAGGCAG
1388	CAUCCAGU	CUGAUGAGGCOGAAAGGCOGAA	AGUCUCCA
1398	UGUCCUGU	CUGAUGAGGCOGAAAGGCOGAA	ACAGCCAG
1402	CAGUUCUC	CUGAUGAGGCOGAAAGGCOGAA	AAGCACAG
1408	GACGCCAC	CUGAUGAGGCOGAAAGGCOGAA	AUCACGAA
1410	GUCCACUC	CUGAUGAGGCOGAAAGGCOGAA	AUAGUUCG
1421	GCCUGGGG	CUGAUGAGGCOGAAAGGCOGAA	AAGUACCC
1425	AGCCAGAG	CUGAUGAGGCOGAAAGGCOGAA	AGGUGGGU
1429	CCUGAGGC	CUGAUGAGGCOGAAAGGCOGAA	ACAAGUAT
1444	CUCCUCCU	CUGAUGAGGCOGAAAGGCOGAA	AGCCUUCU
1455	UCCCUUGU	CUGAUGAGGCOGAAAGGCOGAA	AUACUCCC
1482	CCUGGGGG	CUGAUGAGGCOGAAAGGCOGAA	AGUACCCU
1484	GCAAGAGG	CUGAUGAGGCOGAAAGGCOGAA	ACAGCAGU
1493	UAGUCUCC	CUGAUGAGGCOGAAAGGCOGAA	ACCCAGG

1500	UUGA ¹ CCAU	CUGAUGAGGCOGAAAGGCOGAA	AUUUCACG
1503	GUGGUUGG	CUGAUGAGGCOGAAAGGCOGAA	ACAUUUUC
1506	CCAACAAT	CUGAUGAGGCOGAAAGGCOGAA	ADGACCCA
1509	UACACAGU	CUGAUGAGGCOGAAAGGCOGAA	AUGGUGGC
1518	ACAACGGC	CUGAUGAGGCOGAAAGGCOGAA	ACCAGGAC
1530	ACAUFUAT	CUGAUGAGGCOGAAAGGCOGAA	ACCCAGGU
1533	AAGCCCGC	CUGAUGAGGCOGAAAGGCOGAA	ADGADUAG
1551	UACGAGCA	CUGAUGAGGCOGAAAGGCOGAA	AGGGCCAC
1559	UAAACAGG	CUGAUGAGGCOGAAAGGCOGAA	ACUCCCCA
1563	UGGGAACA	CUGAUGAGGCOGAAAGGCOGAA	AGGUAGGA
1565	GGGGUAA	CUGAUGAGGCOGAAAGGCOGAA	AGGUGUAA
1567	CUGGCGGU	CUGAUGAGGCOGAAAGGCOGAA	ADAGGUGU
1584	UATAUCCU	CUGAUGAGGCOGAAAGGCOGAA	AUCUUCUU
1592	GUAAUUG	CUGAUGAGGCOGAAAGGCOGAA	AUAUCCUG
1599	GCCUUCUG	CUGAUGAGGCOGAAAGGCOGAA	AACUUGUA
1651	GGCUCAGG	CUGAUGAGGCOGAAAGGCOGAA	AGGCGGGG
1661	ACCAGGGC	CUGAUGAGGCOGAAAGGCOGAA	AAGUGCAG
1663	U ¹ UCCAUU	CUGAUGAGGCOGAAAGGCOGAA	ADUUGUUC
1678	CCCAGGCC	CUGAUGAGGCOGAAAGGCOGAA	AGGUUCUC
1680	GACCUUG	CUGAUGAGGCOGAAAGGCOGAA	AGAAGCCG
1681	GAGGCAGG	CUGAUGAGGCOGAAAGGCOGAA	AACAGGCC
1684	GAGAGGUC	CUGAUGAGGCOGAAAGGCOGAA	ACGAGCAG
1690	AAUGUADG	CUGAUGAGGCOGAAAGGCOGAA	AGGUGGGG
1691	GAAGADCG	CUGAUGAGGCOGAAAGGCOGAA	AAGUCCGG
1696	GCGACCAAG	CUGAUGAGGCOGAAAGGCOGAA	ACCAGGAG
1698	UCUCCAGG	CUGAUGAGGCOGAAAGGCOGAA	AUAUCUGA
1737	GCAUCCUG	CUGAUGAGGCOGAAAGGCOGAA	AUGUGAUC
1750	AAUAGGUG	CUGAUGAGGCOGAAAGGCOGAA	AAAUUGAC
1756	AGGACCAAG	CUGAUGAGGCOGAAAGGCOGAA	AGCAGAGG
1787	CCCAGGCC	CUGAUGAGGCOGAAAGGCOGAA	AGGUUCUC
1790	GAGUUGGG	CUGAUGAGGCOGAAAGGCOGAA	ACAGUGUC
1793	GUCCAGGU	CUGAUGAGGCOGAAAGGCOGAA	AGGACCAU
1797	UGGUUUUU	CUGAUGAGGCOGAAAGGCOGAA	AACAGGGA
1802	UCCAGGUA	CUGAUGAGGCOGAAAGGCOGAA	AUCUGAGC
1812	UUUCCCCA	CUGAUGAGGCOGAAAGGCOGAA	ACUCUGUU
1813	ACGAUCAC	CUGAUGAGGCOGAAAGGCOGAA	AAGCCCGC
1825	UACACAGU	CUGAUGAGGCOGAAAGGCOGAA	AUGGUGGC
1837	UACCCUGU	CUGAUGAGGCOGAAAGGCOGAA	AGGUGGGU
1845	GCCCCUCC	CUGAUGAGGCOGAAAGGCOGAA	AGUCCUCU
1856	GCAGGUCA	CUGAUGAGGCOGAAAGGCOGAA	AUUAGGGG
1861	GGACCAUA	CUGAUGAGGCOGAAAGGCOGAA	AGCACADG
1865	CUUGUGUC	CUGAUGAGGCOGAAAGGCOGAA	ACCGGAUA
1868	AUUUAUAT	CUGAUGAGGCOGAAAGGCOGAA	ACUUGUGA
1877	CCUGGGGG	CUGAUGAGGCOGAAAGGCOGAA	AGUACUGU
1901	UGUACCUU	CUGAUGAGGCOGAAAGGCOGAA	AGUUUUAG
1912	UGUCCAUU	CUGAUGAGGCOGAAAGGCOGAA	AUCUGUUC
1922	UAGGCAAU	CUGAUGAGGCOGAAAGGCOGAA	ACUUAUAT
1923	CUAAAGGU	CUGAUGAGGCOGAAAGGCOGAA	AGCGUCCA
1928	UCCAGGUA	CUGAUGAGGCOGAAAGGCOGAA	AUCUGAGC

SUBSTITUTE SHEET (RULE 26)

NUC 37814

1930	CAUCCAGU	CUGAUGAGGCGAAAGGCGGAA	AGUCUCCA
1964	GCUGACAC	CUGAUGAGGCGAAAGGCGGAA	AAAUCCU
1983	CCCAGGCC	CUGAUGAGGCGAAAGGCGGAA	AGGUUCUC
1996	AGCUUGAA	CUGAUGAGGCGAAAGGCGGAA	AGCUUCCA
2005	UAGGCAAU	CUGAUGAGGCGAAAGGCGGAA	ACUACAU
2013	CAUCCCGA	CUGAUGAGGCGAAAGGCGGAA	AGGCAGCG
2015	ACCADUCC	CUGAUGAGGCGAAAGGCGGAA	AUAGGCAG
2020	GUACAGGG	CUGAUGAGGCGAAAGGCGGAA	ACUCAUA
2039	UCGUUUGU	CUGAUGAGGCGAAAGGCGGAA	AUCCUCCG
2040	ACCUCCAG	CUGAUGAGGCGAAAGGCGGAA	AGGUCAGG
2057	AGCCAUUG	CUGAUGAGGCGAAAGGCGGAA	AGGACAG
2061	UAGGUGUA	CUGAUGAGGCGAAAGGCGGAA	AUGGACGC
2071	CCUGAGGC	CUGAUGAGGCGAAAGGCGGAA	ACAAGUAT
2076	UUAGGCTU	CUGAUGAGGCGAAAGGCGGAA	AGGCUACA
2097	ACAUCAAC	CUGAUGAGGCGAAAGGCGGAA	AGAGUUGG
2098	ACCUCCAG	CUGAUGAGGCGAAAGGCGGAA	AGGUCAGG
2115	CAGGACCC	CUGAUGAGGCGAAAGGCGGAA	AGUCCGAA
2128	GAUCAUGG	CUGAUGAGGCGAAAGGCGGAA	ACAGCACU
2130	AGAGGCAG	CUGAUGAGGCGAAAGGCGGAA	AAACAGGC
2145	ACAUCAAC	CUGAUGAGGCGAAAGGCGGAA	AGAGUUGG
2152	AAGUUGUA	CUGAUGAGGCGAAAGGCGGAA	AUUCUCAA
2156	UCAADAAA	CUGAUGAGGCGAAAGGCGGAA	AACUGUCA
2158	AAUUAUA	CUGAUGAGGCGAAAGGCGGAA	AUACAUCA
2159	GAUUAUAT	CUGAUGAGGCGAAAGGCGGAA	AUACAUUC
2160	UGAAUUA	CUGAUGAGGCGAAAGGCGGAA	AAAUACAU
2162	AACAAAGG	CUGAUGAGGCGAAAGGCGGAA	AGGAUUGU
2163	CUUGAAU	CUGAUGAGGCGAAAGGCGGAA	AUUAUAUA
2166	AAUUAUA	CUGAUGAGGCGAAAGGCGGAA	AUACAUCA
2167	GAUUAUAT	CUGAUGAGGCGAAAGGCGGAA	AUACAUUC
2170	UCUGAAU	CUGAUGAGGCGAAAGGCGGAA	AUUAUAUC
2171	UACUCAAU	CUGAUGAGGCGAAAGGCGGAA	AUUAACUG
2173	GAGGACCA	CUGAUGAGGCGAAAGGCGGAA	AUAGCACU
2174	AGCAGGGG	CUGAUGAGGCGAAAGGCGGAA	AAUAGAGA
2175	UGACUCGU	CUGAUGAGGCGAAAGGCGGAA	AAAGAAAU
2176	GUGGUUGG	CUGAUGAGGCGAAAGGCGGAA	ACAUUUUC
2183	UCAADAAA	CUGAUGAGGCGAAAGGCGGAA	AACUGUCA
2185	ACUCAUA	CUGAUGAGGCGAAAGGCGGAA	AUUAACUG
2186	UACUCAAU	CUGAUGAGGCGAAAGGCGGAA	AAUAACUG
2187	GUACUCAA	CUGAUGAGGCGAAAGGCGGAA	AAUAACU
2189	GGGUACUC	CUGAUGAGGCGAAAGGCGGAA	AUUAUAUA
2196	CAAUAAAU	CUGAUGAGGCGAAAGGCGGAA	ACUGUCAG
2198	UGACCUUG	CUGAUGAGGCGAAAGGCGGAA	AGACAUUC
2199	CUGGCADG	CUGAUGAGGCGAAAGGCGGAA	AAGAGUCU
2200	GCCUGGGG	CUGAUGAGGCGAAAGGCGGAA	AAGUACCC
2201	GACCUUGU	CUGAUGAGGCGAAAGGCGGAA	AGAAGCCC
2205	CAGUGGCU	CUGAUGAGGCGAAAGGCGGAA	ACACAAAA
2210	CAUCCAGU	CUGAUGAGGCGAAAGGCGGAA	AGUCUCCA
2220	CCCAGGCC	CUGAUGAGGCGAAAGGCGGAA	AGGUUCUC
2224	AAGGUAGG	CUGAUGAGGCGAAAGGCGGAA	AUGUAUGU

SUBSTITUTE SHEET (RULE 26)

NUC 37815

2226	UGUGGOCU	CUGAUGAGGCOGAAAGGCOGAA	AGGUCCAG
2233	AGUUCUGU	CUGAUGAGGCOGAAAGGCOGAA	AAGCAGCA
2242	ACUACUGA	CUGAUGAGGCOGAAAGGCOGAA	AGCUGUGU
2248	GCGACCAG	CUGAUGAGGCOGAAAGGCOGAA	ACCAGGAG
2254	UUCAGUGU	CUGAUGAGGCOGAAAGGCOGAA	AAUUGGAD
2259	GCAACGUG	CUGAUGAGGCOGAAAGGCOGAA	AUGUGAUC
2260	AGCACCGU	CUGAUGAGGCOGAAAGGCOGAA	AAUGUGAU
2266	AACUUGUA	CUGAUGAGGCOGAAAGGCOGAA	ADCCUGAU
2274	UACADGUU	CUGAUGAGGCOGAAAGGCOGAA	ACCTGCUU
2279	ACCGGUAU	CUGAUGAGGCOGAAAGGCOGAA	ADCTUUCU
2282	ACUCAADA	CUGAUGAGGCOGAAAGGCOGAA	ADAACUGU
2288	CAUUGGAG	CUGAUGAGGCOGAAAGGCOGAA	ACCAGGGC
2291	GUAACUUG	CUGAUGAGGCOGAAAGGCOGAA	AUAUCCUG
2321	ACCGGUAU	CUGAUGAGGCOGAAAGGCOGAA	AUCUUCUU
2338	CCUGUGGA	CUGAUGAGGCOGAAAGGCOGAA	AAGCCCAA
2339	GCCUGGGG	CUGAUGAGGCOGAAAGGCOGAA	AAGUACCC
2341	UGAGCACC	CUGAUGAGGCOGAAAGGCOGAA	ACAGGCCU
2344	GAGAGGUC	CUGAUGAGGCOGAAAGGCOGAA	ACGAGCAG
2358	UGUGGGAG	CUGAUGAGGCOGAAAGGCOGAA	AGGCAGGG
2359	UUCUGUGG	CUGAUGAGGCOGAAAGGCOGAA	AUGGAUGG
2360	CUUCCAGG	CUGAUGAGGCOGAAAGGCOGAA	AACACAAG
2376	AAGAGGAA	CUGAUGAGGCOGAAAGGCOGAA	AGCAGUUC
2377	UAADAGAG	CUGAUGAGGCOGAAAGGCOGAA	AGGAAGUC
2378	UCGUGAAA	CUGAUGAGGCOGAAAGGCOGAA	AAAUAGGC
2379	CGCAAGAG	CUGAUGAGGCOGAAAGGCOGAA	AAGAGCAG
2380	ACUUGUGA	CUGAUGAGGCOGAAAGGCOGAA	AGAAADCA
2382	UGACUUGU	CUGAUGAGGCOGAAAGGCOGAA	AAAGAAAU
2384	CUUGUGUC	CUGAUGAGGCOGAAAGGCOGAA	ACCGGAUA
2399	CGUCCACA	CUGAUGAGGCOGAAAGGCOGAA	AGUAUUUA
2401	GAGGACCA	CUGAUGAGGCOGAAAGGCOGAA	AUAGCACA
2411	UGAAGCAU	CUGAUGAGGCOGAAAGGCOGAA	AGAAAUUG
2417	AACUUGUA	CUGAUGAGGCOGAAAGGCOGAA	AUCCUGAU
2418	AGUUCUGU	CUGAUGAGGCOGAAAGGCOGAA	AAGCAGCA
2425	GAACUCUG	CUGAUGAGGCOGAAAGGCOGAA	AUUAADAA
2426	UAGUCUCC	CUGAUGAGGCOGAAAGGCOGAA	ACCCAGGG
2433	AACUGUCA	CUGAUGAGGCOGAAAGGCOGAA	AACUCUGA
2434	UCGUUUGU	CUGAUGAGGCOGAAAGGCOGAA	AUCCUCCG
2448	GGGGGAAG	CUGAUGAGGCOGAAAGGCOGAA	ACUGUCCA
2449	CGAGGCAG	CUGAUGAGGCOGAAAGGCOGAA	AAGGCUUC
2451	GAGGCAGG	CUGAUGAGGCOGAAAGGCOGAA	AACAGGCC
2452	AGAGGCAG	CUGAUGAGGCOGAAAGGCOGAA	AAACAGGC
2455	AACAAAGG	CUGAUGAGGCOGAAAGGCOGAA	AGGAADGU
2459	UGUGGGAG	CUGAUGAGGCOGAAAGGCOGAA	AGGCAGGG
2460	UUGGGAAC	CUGAUGAGGCOGAAAGGCOGAA	AAGGUAGG
2479	GGCGGUAA	CUGAUGAGGCOGAAAGGCOGAA	AGGUGUAA
2480	GGGADCAC	CUGAUGAGGCOGAAAGGCOGAA	ACGGCGAC
2483	ACAUUGGG	CUGAUGAGGCOGAAAGGCOGAA	ACAAAGGU
2484	GACAUUGG	CUGAUGAGGCOGAAAGGCOGAA	AACAAAGG
2492	UAGGUGGG	CUGAUGAGGCOGAAAGGCOGAA	AGGUGGUC

2504	UAGGAADG	CUGAUGAGGCOOGAAAGGCOOGAA	AUGUAGGU
2508	AAGGUAGG	CUGAUGAGGCOOGAAAGGCOOGAA	AUGUADGU
2509	AAAGGUAG	CUGAUGAGGCOOGAAAGGCOOGAA	AAUGUADG
2510	AAUAGGOG	CUGAUGAGGCOOGAAAGGCOOGAA	AAAUUGGAC
2520	ACAUDGGG	CUGAUGAGGCOOGAAAGGCOOGAA	ACAAAGGU
2521	GACAUUGG	CUGAUGAGGCOOGAAAGGCOOGAA	AACAAAGG
2533	UGAGGGGU	CUGAUGAGGCOOGAAAGGCOOGAA	AAUGCUGU
2540	GGADUACU	CUGAUGAGGCOOGAAAGGCOOGAA	AGCACOGA
2545	AAAGUCCG	CUGAUGAGGCOOGAAAGGCOOGAA	AGCUGCCU
2568	CUGACACA	CUGAUGAGGCOOGAAAGGCOOGAA	AAUCUCUG
2579	CCAGGGCA	CUGAUGAGGCOOGAAAGGCOOGAA	AGUGCAGG
2585	GAGAGGUC	CUGAUGAGGCOOGAAAGGCOOGAA	ACGAGCAG
2588	GGCUGUGG	CUGAUGAGGCOOGAAAGGCOOGAA	AGGAGGCA
2591	CUUCGCAA	CUGAUGAGGCOOGAAAGGCOOGAA	AGGAAGAG
2593	AGCAGGGG	CUGAUGAGGCOOGAAAGGCOOGAA	AAUAGAGA
2596	GCGACGAG	CUGAUGAGGCOOGAAAGGCOOGAA	ACCAGGAG
2601	GAGGACCA	CUGAUGAGGCOOGAAAGGCOOGAA	AUAGCACA
2602	ACAAOGGC	CUGAUGAGGCOOGAAAGGCOOGAA	ACCAGGAC
2607	CCUGGUGA	CUGAUGAGGCOOGAAAGGCOOGAA	ACUCCAC
2608	UCCACCGG	CUGAUGAGGCOOGAAAGGCOOGAA	AGCUAAAG
2609	CAUCCAGU	CUGAUGAGGCOOGAAAGGCOOGAA	AGUCUCCA
2620	AACUGUCA	CUGAUGAGGCOOGAAAGGCOOGAA	AAUCUGA
2626	AGCAGCAC	CUGAUGAGGCOOGAAAGGCOOGAA	ACUGAGAG
2628	GGAGCOGA	CUGAUGAGGCOOGAAAGGCOOGAA	AAUUGUA
2635	GUGAAUUG	CUGAUGAGGCOOGAAAGGCOOGAA	AUCUGUGA
2640	UGGAUGGA	CUGAUGAGGCOOGAAAGGCOOGAA	ACCUAGGC
2641	AAUGUADG	CUGAUGAGGCOOGAAAGGCOOGAA	AGGUGGGG
2642	AGAGGCAG	CUGAUGAGGCOOGAAAGGCOOGAA	AAACAGGC
2653	AGCACCCU	CUGAUGAGGCOOGAAAGGCOOGAA	ACCUUGGG
2659	GCUUGCAG	CUGAUGAGGCOOGAAAGGCOOGAA	ACCCUUCU
2689	AGCUUCAG	CUGAUGAGGCOOGAAAGGCOOGAA	ACCCUAGU
2691	AGUCCUCU	CUGAUGAGGCOOGAAAGGCOOGAA	AGGCCUGA
2700	CCUGGGGG	CUGAUGAGGCOOGAAAGGCOOGAA	AGUACCCU
2704	UAGGUGGG	CUGAUGAGGCOOGAAAGGCOOGAA	AGGUGGUC
2711	ACCUUCCU	CUGAUGAGGCOOGAAAGGCOOGAA	AGGUAGGG
2712	CACCUUCC	CUGAUGAGGCOOGAAAGGCOOGAA	AAGGUAGG
2721	ACCCGUUU	CUGAUGAGGCOOGAAAGGCOOGAA	AUCUUUCC
2724	CRAACCCG	CUGAUGAGGCOOGAAAGGCOOGAA	AUGADCUU
2744	CCUGCAGG	CUGAUGAGGCOOGAAAGGCOOGAA	AUCCACCC
2750	GGUUUUUA	CUGAUGAGGCOOGAAAGGCOOGAA	ACAGGGAC
2759	CCACUUGA	CUGAUGAGGCOOGAAAGGCOOGAA	AGUUUGUC
2761	GGAAGAUU	CUGAUGAGGCOOGAAAGGCOOGAA	AAAGUCCG
2765	AGGCCGCA	CUGAUGAGGCOOGAAAGGCOOGAA	AGCAAAAG
2769	GCAGGGGU	CUGAUGAGGCOOGAAAGGCOOGAA	AUAGAGAA
2797	UUGACCAU	CUGAUGAGGCOOGAAAGGCOOGAA	AUUUCACG
2803	GUUCUGUG	CUGAUGAGGCOOGAAAGGCOOGAA	AGCAUGAG
2804	AGUUCUGU	CUGAUGAGGCOOGAAAGGCOOGAA	AAGCAUGA
2813	AGGGUCAG	CUGAUGAGGCOOGAAAGGCOOGAA	AUGGGAGC
2815	GGAAGAUU	CUGAUGAGGCOOGAAAGGCOOGAA	AAAGUCCG

2821 ACCUCCAG CUGAUGAGGCOGAAAGGCOGAA AGGUCAGG
2822 GGAGCUGA CUGAUGAGGCOGAAAGGCOGAA AAGUUGUA
2823 UGGGAGCU CUGAUGAGGCOGAAAGGCOGAA AAAAGUUG
2829 GGAIACCU CUGAUGAGGCOGAAAGGCOGAA AGCACOGA
2837 GGGGGAAG CUGAUGAGGCOGAAAGGCOGAA ACCUGUG
2840 UGCGCUGG CUGAUGAGGCOGAAAGGCOGAA AGGGGUGC
2847 AGGGGGU CUGAUGAGGCOGAAAGGCOGAA AGGGGUA
2853 CUAGUCCG CUGAUGAGGCOGAAAGGCOGAA AGAUCOGA
2860 UUCGAGG CUGAUGAGGCOGAAAGGCOGAA ACACAAGA
2872 UGAGCAC CUGAUGAGGCOGAAAGGCOGAA ACAGGCCC
2877 GGCGCUGG CUGAUGAGGCOGAAAGGCOGAA AGACUCCA
2899 AAAGUCCG CUGAUGAGGCOGAAAGGCOGAA AGCUGCCU
2900 AGAGAAGG CUGAUGAGGCOGAAAGGCOGAA AGUCAGCC
2904 AAGAGGAA CUGAUGAGGCOGAAAGGCOGAA AGCAGUUC
2905 AGAGAAGG CUGAUGAGGCOGAAAGGCOGAA AGUCAGCC
2906 UUAADTAA CUGAUGAGGCOGAAAGGCOGAA ACADCAAC
2907 CGCAAGAG CUGAUGAGGCOGAAAGGCOGAA AAGAGCAG
2908 AADUADA CUGAUGAGGCOGAAAGGCOGAA ADACADCA
2909 AAGAGGAA CUGAUGAGGCOGAAAGGCOGAA AGCAGUUC
2910 GUAADTAA CUGAUGAGGCOGAAAGGCOGAA AAGGAAGU
2911 GGGUAAUA CUGAUGAGGCOGAAAGGCOGAA AGAAGGAA
2912 UGAADUAA CUGAUGAGGCOGAAAGGCOGAA AAADACAU
2913 CUGGGAAC CUGAUGAGGCOGAAAGGCOGAA AAUACACA
2914 UCUGAATU CUGAUGAGGCOGAAAGGCOGAA ADAAADAC
2915 CUCUGAAT CUGAUGAGGCOGAAAGGCOGAA AAUAAUA
2916 CUUCGCAA CUGAUGAGGCOGAAAGGCOGAA AGGAAGAG
2917 GUCUUCGC CUGAUGAGGCOGAAAGGCOGAA AGAGGAAG
2918 UGACUCGU CUGAUGAGGCOGAAAGGCOGAA AAAGAAAU
2919 CAGUGGCU CUGAUGAGGCOGAAAGGCOGAA ACACAAA
2931 GGCAGGG CUGAUGAGGCOGAAAGGCOGAA ACACCAUC
2933 GGUGCUGG CUGAUGAGGCOGAAAGGCOGAA AGACUCCA
2941 GCCUGGGG CUGAUGAGGCOGAAAGGCOGAA AAGUACUG
2951 GUCAGAGG CUGAUGAGGCOGAAAGGCOGAA AGCADGGU
2952 GAAGAUCC CUGAUGAGGCOGAAAGGCOGAA AAGUCCGG
2955 CCAUGUCA CUGAUGAGGCOGAAAGGCOGAA AGGAAGCA
2956 AUUGAUTC CUGAUGAGGCOGAAAGGCOGAA AAGGAAG
2961 CAGUGGCU CUGAUGAGGCOGAAAGGCOGAA ACACAAA
2962 CUGGGAAC CUGAUGAGGCOGAAAGGCOGAA AAUACACA
2965 ACUUAATU CUGAUGAGGCOGAAAGGCOGAA ADUCAAAG
2966 AGCUUGAA CUGAUGAGGCOGAAAGGCOGAA AGCUUCCA
2969 UAAAACUU CUGAUGAGGCOGAAAGGCOGAA ADUGAUTC
2975 AGCUUGAA CUGAUGAGGCOGAAAGGCOGAA AGCUUCCA
2976 CAGGUGAG CUGAUGAGGCOGAAAGGCOGAA ACCUAUA
2977 UCAGCUUG CUGAUGAGGCOGAAAGGCOGAA AGAGCUUC

Table 11: Human IL-5 HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	ADGCACU U UCUUUGC	245	AAGAAAU C UUUCAGG
9	UGCACUU U CUUUGGC	247	GAAAUUU U UCAGGGA
10	GCACUUU C UUUGGCA	248	AAAUUUU U CAGGGAA
12	ACUUCUU U UGCCAAA	249	AADUUUU C AGGGAAU
13	CUUUCUU U GCGAAAG	257	AGGGAAU A GGCACAC
36	AGAACGU U UCAGAGC	273	GGAGAGU C AAACUGU
37	GAAGGUU U CAGAGGC	291	AGGGGUU A CUGUGGA
38	AACGUUU C AGAGCCA	305	AAAGACU A UUCAAAA
56	GGAGGUU U CUGCAUU	307	AGACUAA U CAAAAAC
57	GAUGGUU C UGCAUUU	308	GACUAAU C AAAAACU
63	UCUGCAU U UGAGUUU	316	AAAAACU U GUCCUUA
64	CUGCAUU U GAGUUUG	319	AACUUGU C CUUAAUA
69	UUUGAGU U UGCUAGC	322	UUGUCCU U AAUAAG
70	UUGAGUU U GCUAGCU	323	UGUCCUU A AUAAAGA
74	GUUUGCU A GCUUUG	326	CCUAAAU A AAGAAAU
78	GCUAGCU C UUGGAGC	334	AAGAAAU A CAUUGAC
80	UAGGUUU U GGAGGUG	338	AUAACAU U GACGGCC
91	GCUUGCU A CGUGUAA	380	GGAGAGU A AACCAAU
97	UACGUGU A UGCCAUC	388	AACCAAU U CUUAGAC
104	ADGCCAU C CCGACAG	389	ACCAAUU C CUAGACU
116	CAGAAAU U CCGACAA	392	AAUCCUU A GACUACC
117	AGAAAUU C CCGACAG	397	CUAGACU A CCUGCAA
130	AGUGCAU U GGUGAAA	409	CAAGAGU U UCUUGGU
145	GAGACUU U GGCACUG	410	AAGAGUU U CUUGGUG
155	CACUGCU U UCUACUC	411	AGAGUUU C UUGGUGU
156	ACUGGUU U CUACUCA	413	AGUUUCU U GGUGUAA
157	CUGGUUU C UACUCAU	419	UUGGUGU A AUGAACA
159	GCUUUCU A CUCAUUG	437	AGUGGAU A AUAGAAA
162	UUUACU C AUUGAAC	440	GGAUAAU A GAAAGUU
165	UACUCAU C GAACUCU	447	AGAAAGU U GAGACUA
171	UGGAACU C UGCUGAU	454	UGAGACU A AACUGGU
179	UGCUGAU A GCGAAG	462	AACUGGU U UGUUGCA
192	UGAGACU C UGAGGAU	463	ACUGGUU U GUUGCAG
200	UGAGGAU U CCUGUUC	466	GGUUUGU U GCAGCCA
201	GAGGAUU C CUGUUC	479	CAAAGAU U UUGGAGG
206	UUCCUGU U CCUGUAC	480	AAAGAUU U UGGAGGA
207	UCCUGUU C CUGUACA	481	AAGAUUU U GGAGGAG
212	UUCCUGU A CAUAAAA	497	AGGACAU U UUAUCUG
216	UGUACAU A AAAAUCA	498	GGACAUU U UACUGCA
222	UAAAAAU C ACCAACU	499	GACAUUU U ACUGCAG

500	ACADUUU A CUGCAGU	684	UACUUUU U UCUUADU
531	AAAGAGU C AGGCCUU	685	ACUUUUU U CUUADUU
538	CAGGCCU U AAUUUUC	686	CUUUUUU C UUAUUUA
539	AGGCCUU A AUUUUCA	688	UUUUUUU U AUUUUAC
542	CCUUAAU U UUCAAUU	689	UUUUUUU A UUUUAAU
543	CUUAAUU U UCAAUUU	691	UUCUUUU U UAAUUUA
544	UUAAUUU U CAUAUUU	692	UCUUUUU U AACUUUA
545	UAUUUUU C AAUAUUU	693	CUUAAUU A ACUUUAC
549	UUUCAUU A UAUUUUA	697	UUUAAUU U AACUUUC
551	UCAAUUU A AUUUUAC	698	UUAAUUU A ACUUUUC
554	AAUAUUU U UAACUUU	703	UUAAUUU U CUGUUUA
555	UAUAUUU U AACUUUA	704	UAACUUU C UGUUUUA
556	AUAUUUU A ACUUUAG	708	AUUUUUU A AAUUUUC
560	UUUAUUU U CAGAGGU	715	AAAUUUU C UGUUUAC
561	UUAAUUU C AGAGGUA	719	UGUUUUU U AACUUUA
573	GGAAAGU A AAUAUUU	720	GUUUUUU A ACUUUAA
577	AGUAAUU A UUUCAGG	724	GUUAAUU U AAUAUUU
579	UAUAUUU U UCAGGUA	725	UUAAUUU A AAUAUUU
580	AAUAUUU U CAGGUAU	728	ACUUUAA A GUUUUUA
581	AAUAUUU C AGGUAUU	731	UAUAUUU A UUUAUUU
588	CAGGUAU A CUGAAC	733	AAUAUUU U UAUAUUU
597	UGAACUU U UGUAUA	734	UAUAUUU U AAUAUUU
598	GACACUU U GUUAUA	735	AGUAUUU A UGUAUUU
611	AAAGUAU A AAUUUUC	745	AAAUUUU U AUAUUU
616	AUAUUUU U CUUUAAA	746	AAUUGUU A AGUAUUU
617	UAUUUUU C UUAUUU	752	UAUAUUU U UGUUUUA
619	AAAUUUU U AAUAUUU	753	AAUAUUU U GUUUUUA
620	AAUUUUU A AAUAUUU	757	AUUUUUU A AAUAUUU
625	UUUUUUU A UAUUUCA	761	GUUUUUU U AGUAUUU
627	AAUAUUU A UUUCAGU	762	GUUUUUU A GUUUUUA
629	AAUAUUU U UCAGUAU	765	AAUAUUU A UUUAUUU
630	AAUAUUU U CAGUAUU	767	UUUAUUU U UAUAUUU
631	UAUAUUU C AGUAUUU	768	UAUAUUU U AAUAUUU
636	UUCAUUU A UCAGUAU	769	AGUAUUU A UUUAUUU
638	CAGUAUU C AGUAUUU	771	UAUAUUU U UAUAUUU
644	UCAGUAU C AUUGAAG	772	AUUUUUU U AAUAUUU
647	GAUUUUU U GAUAUUU	773	UUUAUUU A AAUAUUU
653	UUUAUUU A UUUUUUU	778	UUUAUUU U AAUAUUU
655	GAUAUUU U UUUUUUU	779	UAUAUUU A UGUUUUU
656	AAUAUUU U UUUUUUU	783	GUUUUUU U GUUUUUU
657	AGUAUUU U UUUUUUU	788	GUUUUUU U CUUUUUU
658	GUUUUUU C CUUUUUU	789	UUUUUUU C UAUAUUU
661	UUUUUUU C CAGGUAU	791	GUUUUUU A AAUAUUU
672	GUUUUUU U GAUAUUU	794	UUUUUUU A AAUAUUU
676	AAUAUUU A UAUAUUU	805	CAUAUUU A GACUAUU
678	UUUAUUU A CUUUUUU		
681	AAUAUUU U UUUUUUU		
682	UAUAUUU U UUUUUUU		

SUBSTITUTE SHEET (RULE 26)

NUC 37820

Table 12: Human IL-5 HH Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
8	GCAAAGA CUGADGAGGCGGAAAGGCGGAA AGUGCAU
9	GGCAAAG CUGADGAGGCGGAAAGGCGGAA AAGUGCA
10	UGGCAAA CUGADGAGGCGGAAAGGCGGAA AAAGUGC
12	UUUGGCA CUGADGAGGCGGAAAGGCGGAA AGAAAGU
13	CUUUGGC CUGADGAGGCGGAAAGGCGGAA AAGAAAG
36	GCUCUGA CUGADGAGGCGGAAAGGCGGAA ACGUUCU
37	GGCUCUG CUGADGAGGCGGAAAGGCGGAA AACGUUC
38	UGGCUUC CUGADGAGGCGGAAAGGCGGAA AAACGUU
56	AADGCAG CUGADGAGGCGGAAAGGCGGAA AGCAUCC
57	AAAUCCA CUGADGAGGCGGAAAGGCGGAA AAGCAUC
63	AAACUCA CUGADGAGGCGGAAAGGCGGAA AUGCAGA
64	CAAACUC CUGADGAGGCGGAAAGGCGGAA AADGCAG
69	GCUAGCA CUGADGAGGCGGAAAGGCGGAA ACUCAAA
70	AGCUAGC CUGADGAGGCGGAAAGGCGGAA AACUCAA
74	CAAGAGC CUGADGAGGCGGAAAGGCGGAA AGCAAAC
78	GCUCCAA CUGADGAGGCGGAAAGGCGGAA AGCUAGC
80	CAGCUCC CUGADGAGGCGGAAAGGCGGAA AGAGCUA
91	AUACAGC CUGADGAGGCGGAAAGGCGGAA AGGCAGC
97	GADGGCA CUGADGAGGCGGAAAGGCGGAA ACACGUA
104	CUGUGGG CUGADGAGGCGGAAAGGCGGAA AUGGCCAU
116	UUGUGGG CUGADGAGGCGGAAAGGCGGAA AUUUCUG
117	CUUGUGG CUGADGAGGCGGAAAGGCGGAA AAUUCUC
130	UUUCACC CUGADGAGGCGGAAAGGCGGAA AUGCACU
145	CAGUGCC CUGADGAGGCGGAAAGGCGGAA AGGUCCU
155	GAGUAGA CUGADGAGGCGGAAAGGCGGAA AGCAGUG
156	UGAGUAG CUGADGAGGCGGAAAGGCGGAA AAGCAGU
157	AUGAGUA CUGADGAGGCGGAAAGGCGGAA AAAGCAG
159	CGADGAG CUGADGAGGCGGAAAGGCGGAA AGAAAGC
162	GUUCGAU CUGADGAGGCGGAAAGGCGGAA AGUAGAA
165	AGAGUUC CUGADGAGGCGGAAAGGCGGAA AUGAGUA
171	AUCAGCA CUGADGAGGCGGAAAGGCGGAA AGUUGCA
179	CAUUGGC CUGADGAGGCGGAAAGGCGGAA AUCAGCA
192	AUCCUCA CUGADGAGGCGGAAAGGCGGAA AGUCUCA
200	GAACAGG CUGADGAGGCGGAAAGGCGGAA AUCCUCA
201	GGAACAG CUGADGAGGCGGAAAGGCGGAA AAUCCUC
206	GUACAGG CUGADGAGGCGGAAAGGCGGAA ACAGGAA
207	UGUACAG CUGADGAGGCGGAAAGGCGGAA AACAGGA
212	UUUUADG CUGADGAGGCGGAAAGGCGGAA ACAGGAA
216	UGAUUUU CUGADGAGGCGGAAAGGCGGAA AUGUACA
222	AGUUGGU CUGADGAGGCGGAAAGGCGGAA AUUUUUA
245	CCUGAAA CUGADGAGGCGGAAAGGCGGAA AUUUCUU

SUBSTITUTE SHEET (RULE 26)

NUC 37821

247 UCCCTGA CUGAUGAGGCGAAAGGCGGAA AGAUUUC
248 UCCCTUG CUGAUGAGGCGAAAGGCGGAA AAGAUUU
249 AUUCCCU CUGAUGAGGCGAAAGGCGGAA AAAGAUU
257 GUGUGCC CUGAUGAGGCGAAAGGCGGAA AUUCCCU
273 ACAGUUU CUGAUGAGGCGAAAGGCGGAA ACUCUCC
291 UCCACAG CUGAUGAGGCGAAAGGCGGAA ACCCCCU
305 UUUUGAA CUGAUGAGGCGAAAGGCGGAA AGUCUUU
307 GUUUUUG CUGAUGAGGCGAAAGGCGGAA ADAGUCU
308 AGUUUUU CUGAUGAGGCGAAAGGCGGAA AAUAGUC
316 UAAGGAC CUGAUGAGGCGAAAGGCGGAA AGUUUUU
319 UAUUAG CUGAUGAGGCGAAAGGCGGAA ACAAGUU
322 CUUUUUU CUGAUGAGGCGAAAGGCGGAA AGGACAA
323 UCUUUAU CUGAUGAGGCGAAAGGCGGAA AAGGACA
326 ADUUCUU CUGAUGAGGCGAAAGGCGGAA ADUAAGG
334 GUCAADG CUGAUGAGGCGAAAGGCGGAA ADUUCUU
338 GGCCGUC CUGAUGAGGCGAAAGGCGGAA AUGUAUU
380 AUUGGUU CUGAUGAGGCGAAAGGCGGAA ACUCUCC
388 GUUUAGG CUGAUGAGGCGAAAGGCGGAA AUUGGUU
389 AGUCUAG CUGAUGAGGCGAAAGGCGGAA AAUUGGU
392 GGUAGUC CUGAUGAGGCGAAAGGCGGAA AGGAUUU
397 UUGCAGG CUGAUGAGGCGAAAGGCGGAA AGUCUAG
409 ACCAAGA CUGAUGAGGCGAAAGGCGGAA ACUCUUG
410 CACCAAG CUGAUGAGGCGAAAGGCGGAA AACUCUU
411 ACACCAA CUGAUGAGGCGAAAGGCGGAA AAACUCU
413 UUACACC CUGAUGAGGCGAAAGGCGGAA AGAAACT
419 UGUUCAU CUGAUGAGGCGAAAGGCGGAA ACACCAA
437 UUUCUAU CUGAUGAGGCGAAAGGCGGAA AUCCACU
440 AACUUUC CUGAUGAGGCGAAAGGCGGAA AUUADCC
447 UAGUCUC CUGAUGAGGCGAAAGGCGGAA ACUUUCU
454 ACCAGUU CUGAUGAGGCGAAAGGCGGAA AGUCUCA
462 UGCAACA CUGAUGAGGCGAAAGGCGGAA ACCAGUU
463 CUGCAAC CUGAUGAGGCGAAAGGCGGAA AACCAGU
466 UGGCUGC CUGAUGAGGCGAAAGGCGGAA ACAAAAC
479 CCUCCAA CUGAUGAGGCGAAAGGCGGAA AUCUUUG
480 UCCUCCA CUGAUGAGGCGAAAGGCGGAA AAUCUUU
481 CUCCUCC CUGAUGAGGCGAAAGGCGGAA AAADCUU
497 GCAGUAA CUGAUGAGGCGAAAGGCGGAA AUGUCCU
498 UGCAGUA CUGAUGAGGCGAAAGGCGGAA AAUGUCC
499 CUGCAGU CUGAUGAGGCGAAAGGCGGAA AAADGUC
500 ACUGCAG CUGAUGAGGCGAAAGGCGGAA AAAADGU
531 AAGGCGU CUGAUGAGGCGAAAGGCGGAA ACUCUUU
538 GAAAUUU CUGAUGAGGCGAAAGGCGGAA AGGCCUG
539 UGAAAUU CUGAUGAGGCGAAAGGCGGAA AAGGCCU
542 UAUUGAA CUGAUGAGGCGAAAGGCGGAA AUUAAGG
543 AAUUGAA CUGAUGAGGCGAAAGGCGGAA AAUUAAG
544 UAUUUGU CUGAUGAGGCGAAAGGCGGAA AAUUAAG
545 UUAUUAU CUGAUGAGGCGAAAGGCGGAA AAAAUUA
549 UAAAUUA CUGAUGAGGCGAAAGGCGGAA AUUGAAA
551 GUUAAAU CUGAUGAGGCGAAAGGCGGAA AUUAUGA

554 GAAGTUA CUGAUGAGGCOGAAAGGCOGAA AUUAUAU
555 UGAAGUU CUGAUGAGGCOGAAAGGCOGAA AAUUAUA
556 CUGAAGU CUGAUGAGGCOGAAAGGCOGAA AAAUUAU
560 CUCUCUG CUGAUGAGGCOGAAAGGCOGAA AGUUAUA
561 UCCUCU CUGAUGAGGCOGAAAGGCOGAA AAGUUAU
573 AAUAUAU CUGAUGAGGCOGAAAGGCOGAA ACUUAUC
577 CCUGAAA CUGAUGAGGCOGAAAGGCOGAA AUUAUAU
579 UGCCUGA CUGAUGAGGCOGAAAGGCOGAA AUUAUAU
580 AUGCCUG CUGAUGAGGCOGAAAGGCOGAA AAUAUAU
581 UAUCCU CUGAUGAGGCOGAAAGGCOGAA AAUAUAU
588 GUGUCAG CUGAUGAGGCOGAAAGGCOGAA AUGCCUG
597 UCUGGCA CUGAUGAGGCOGAAAGGCOGAA AGUGUCA
598 UUCUGGC CUGAUGAGGCOGAAAGGCOGAA AAGUGUC
611 AGAUAUU CUGAUGAGGCOGAAAGGCOGAA AUGCUUU
616 UUUUAAG CUGAUGAGGCOGAAAGGCOGAA AUUUUAU
617 AUUUUAU CUGAUGAGGCOGAAAGGCOGAA AAUUUAU
619 AAUAUAU CUGAUGAGGCOGAAAGGCOGAA AGAUAUU
620 UAUAUAU CUGAUGAGGCOGAAAGGCOGAA AAGAUAU
625 UGAUAUA CUGAUGAGGCOGAAAGGCOGAA AUUUUAU
627 UCUGAAA CUGAUGAGGCOGAAAGGCOGAA AUUAUAU
629 UAUCUGA CUGAUGAGGCOGAAAGGCOGAA AUUAUAU
630 AUUAUCU CUGAUGAGGCOGAAAGGCOGAA AAUAUAU
631 GAUAUCU CUGAUGAGGCOGAAAGGCOGAA AAUAUAU
636 AUUCUGA CUGAUGAGGCOGAAAGGCOGAA AUUCUGA
638 UGAUUCU CUGAUGAGGCOGAAAGGCOGAA AUUAUCU
644 CUUCAAU CUGAUGAGGCOGAAAGGCOGAA AUUCUGA
647 ADACUUC CUGAUGAGGCOGAAAGGCOGAA AUGAUUC
653 AGGAAAA CUGAUGAGGCOGAAAGGCOGAA ACUUCAA
655 GGAGGAA CUGAUGAGGCOGAAAGGCOGAA AUACUUC
656 UGGAGGA CUGAUGAGGCOGAAAGGCOGAA AAUAUCU
657 CUGGAGG CUGAUGAGGCOGAAAGGCOGAA AAUAUCU
658 CCUGGAG CUGAUGAGGCOGAAAGGCOGAA AAAUAUC
661 UUGCCUG CUGAUGAGGCOGAAAGGCOGAA AGGAAAA
672 GUUAUUC CUGAUGAGGCOGAAAGGCOGAA AUUUUUC
676 AAAAGUA CUGAUGAGGCOGAAAGGCOGAA AUCAUAU
678 AAAAAAG CUGAUGAGGCOGAAAGGCOGAA AUUAUCA
681 AAGAAAA CUGAUGAGGCOGAAAGGCOGAA AGUAUAU
682 UAAGAAA CUGAUGAGGCOGAAAGGCOGAA AAGUADA
683 AUAGAAA CUGAUGAGGCOGAAAGGCOGAA AAAGUAU
684 AAUAAGA CUGAUGAGGCOGAAAGGCOGAA AAAAGUA
685 AAAUAAG CUGAUGAGGCOGAAAGGCOGAA AAAAAGU
686 UAAAUAA CUGAUGAGGCOGAAAGGCOGAA AAAAAAG
688 GUUAAAU CUGAUGAGGCOGAAAGGCOGAA AGAAAAA
689 AGUUAUA CUGAUGAGGCOGAAAGGCOGAA AAGAAAA
691 UAAGUUA CUGAUGAGGCOGAAAGGCOGAA AUUAAGA
692 UUAAGUU CUGAUGAGGCOGAAAGGCOGAA AAUAAGA
693 GUUAAGU CUGAUGAGGCOGAAAGGCOGAA AAUAAGU
697 GAUUGUU CUGAUGAGGCOGAAAGGCOGAA AGUUAUA
698 AGAUGUU CUGAUGAGGCOGAAAGGCOGAA AAGUUAU

703	UUUACAG	CUGAUGAGGCGAAAGGCGGAA	AUGUUA
704	UUUUACA	CUGAUGAGGCGAAAGGCGGAA	AAUGUUA
708	GACAUUU	CUGAUGAGGCGAAAGGCGGAA	ACAGAUU
715	GUUACA	CUGAUGAGGCGAAAGGCGGAA	ACAUUU
719	UUAGUU	CUGAUGAGGCGAAAGGCGGAA	ACAGACA
720	AUUAGU	CUGAUGAGGCGAAAGGCGGAA	AACAGAC
724	UACUUAU	CUGAUGAGGCGAAAGGCGGAA	AGUUAAC
725	AUACUUAU	CUGAUGAGGCGAAAGGCGGAA	AAGUUA
728	UAAAUAC	CUGAUGAGGCGAAAGGCGGAA	AUUAAGU
731	UCAUAA	CUGAUGAGGCGAAAGGCGGAA	ACUUAU
733	UUUUAU	CUGAUGAGGCGAAAGGCGGAA	AUACUUAU
734	AUUUAU	CUGAUGAGGCGAAAGGCGGAA	AUUAUUA
735	CAUUUA	CUGAUGAGGCGAAAGGCGGAA	AAUUAU
745	AUUUAU	CUGAUGAGGCGAAAGGCGGAA	ACCAUUA
746	AAUUUAU	CUGAUGAGGCGAAAGGCGGAA	AACCAUUA
752	UUUUAU	CUGAUGAGGCGAAAGGCGGAA	AUUUAU
753	AUUUAU	CUGAUGAGGCGAAAGGCGGAA	AUUUAU
757	ACUUAU	CUGAUGAGGCGAAAGGCGGAA	ACCAUUA
761	AAUUAU	CUGAUGAGGCGAAAGGCGGAA	AUUUAU
762	UAAUUA	CUGAUGAGGCGAAAGGCGGAA	AUUUAU
765	AAUUAU	CUGAUGAGGCGAAAGGCGGAA	ACUUAU
767	UUUUAU	CUGAUGAGGCGAAAGGCGGAA	AUUAUUA
768	AUUUAU	CUGAUGAGGCGAAAGGCGGAA	AUUUAU
769	CAUUAU	CUGAUGAGGCGAAAGGCGGAA	AAUUAU
771	AACUUA	CUGAUGAGGCGAAAGGCGGAA	AUUUAU
772	UAACUUA	CUGAUGAGGCGAAAGGCGGAA	AUUUAU
773	AUAUUA	CUGAUGAGGCGAAAGGCGGAA	AAUUAU
778	ACAACU	CUGAUGAGGCGAAAGGCGGAA	ACUUAU
779	CACAUA	CUGAUGAGGCGAAAGGCGGAA	AACUUA
783	AGAACAC	CUGAUGAGGCGAAAGGCGGAA	ACUUAU
788	UUUUAU	CUGAUGAGGCGAAAGGCGGAA	ACUUAU
789	UUUUAU	CUGAUGAGGCGAAAGGCGGAA	AACUUA
791	GUUUUAU	CUGAUGAGGCGAAAGGCGGAA	AGAACAC
794	UUUGUUU	CUGAUGAGGCGAAAGGCGGAA	AUUUAU
805	AGUUUUC	CUGAUGAGGCGAAAGGCGGAA	AUUUUAU

Table 13: Mouse IL-5 HH Ribozyme Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	cGQuCUU c CUUUGCu	253	AGGGgcU A GaCuuAC
11	uCUUcCU U UGQuAA	259	UagACAU a CUGaAgA
12	CUUcCUU U GQuAAG	269	GaAGAAU C AAACUGU
36	GAAgacU U CAGAGuC	269	GaAGAAU c AAaCugU
36	GaAgAcU u cAgAGUc	269	GAgaAU c aAAcUgU
37	AAgaqU C AGAGuCA	287	uGGGGGU A CUUGGA
43	UcaGaGU c AUGAgAA	301	AAAugCU A UUCcAAA
58	GGAUGCU U CUGCAcU	301	AAAugCU a UUCcAAa
59	GAUGCUU C UGCAcUU	303	AUGCUAU u CCaAaAc
59	gAUGcUU c uGcAcUU	303	AugCUAU U CcAAAAC
66	CUGCAcU U GAGUgUu	304	ugCUAUU C cAAAACC
82	UgAcucU c aGcUGUG	315	AACcUGU C aUUAADA
91	GcUgUGU c uggGCCA	318	cUGUCaU U AAUAAG
112	ugGagAU U CCCAugA	319	UGUCaUU A AUAAAG
113	gGagAUU C CCAugAG	322	CaUUAU A AAGAAAU
141	GAGACCU U GaCACaG	330	AAGAAAU A CAUGAC
141	GAgACcU U GaCAcAg	334	AAUACAU U GACcGCC
158	gUCcgCU C AcOGAgC	334	AAUaCaU u GACcgCC
167	cCGAgCU C UGuUGAc	384	AggCAGU U CCUGGau
196	UGAGGcU U CCUGUcC	385	ggCagUU C CUgGauU
197	GAGGcUU C CUGUcCC	393	CUgGauU A CCUGCA
197	gAGGCUU c CUGUcCC	405	CAAGAGU U cCUUGGU
202	UUCcUGU c CCUacuC	406	AAGAGUU c CUUGGUG
202	UUCcUGU c CcUAcuc	409	AGUUCcU U GGUGUGA
206	UGUCcCU a cuCaUAA	481	UcaCAAU u UAAGUUA
212	UACUCAU a aAAaUCA	482	cAcAAUU U AAgUUaA
212	UacuCAU A AAAAUCA	483	AcAAUUU A AgUUaAa
218	UaaAaaU c aCcAGCU	483	AcAAUUU a aGUUAaa
218	UAAAAAU C ACCAgCU	495	AAAUUGU c AAcAgAU
218	uAAAAAU c acCAgCU	553	GCUGuuU c CaUUUAU
232	uaUGCAU U GGaGAAA	557	UuUcCAU U UauaUUU
241	gAGAAAU C UUUCAGG	564	UUauAUU u aUgUCCU
241	gAgAaAU c UUucAGG	564	UUauAUU u AugUcCU
241	gagAAAU c UUUCAGG	565	uaUAUUU a ugUCCuG
241	gAgAaAU c UUUCAGg	565	UAUAUUU a UgUCCUg
243	gaAAucU U UCAGgGg	569	UUuAUGU c cUGUaGU
243	GAAAUcU U UCAGGGg	569	uUUUUGU c cUGUaGU
244	AAAUCCU U CAGGGgc	613	AAAGUGU u uzaCCUU
245	AAUCUUU C AGGGgcU	614	AAgUGuU u aACcUUU

620	UUAACcU u uDuGOAU	1407	cCAgUUU A CUcCAGg
793	caAGgCU u UGuGcAU	1407	ccAgUUU a CUCCAGG
816	CUGagUU a UACUCcc	1410	gUUUaCU C CAGGaAA
818	GAgUUAU a cUCCcuC	1434	ADgCUUU U aUuUaAU
825	ACUcCcu c CccCUCA	1434	aUgcUuU U AUUUAAu
825	aCUccCU c CcCcUCA	1434	aUgcUuU u AuUUAAU
839	AuCcucU U cGUUGCA	1435	UgCUUUU a UuUaAUU
840	uCcucUU c GUUGCAu	1435	ugcUUUU a uUUaAUU
863	cAAGUAU U cCAGGCU	1438	UuUUUAU U AAuUcug
864	AAgUAUU c CAGGCUg	1438	uUUUAUU U AAUAucUg
864	AAGUUAU c caggCUg	1439	UUUAUUU A ADucUgU
913	gAaCUUU U GAucCaG	1443	UUUaAuU c UGuaAGA
917	UcUaggU c CAGAUgG	1447	ADUCUGU A AgADGUu
957	UUagcAU c CUUcUc	1458	ugUUcaU a UUAUUUA
960	GCAuccU u UcUcUA	1458	ugUUcaU A uUAUUUA
960	GcaUcCU u uCUUcUA	1460	UcAUUAU u AUUUAug
962	ADcCUuU c UCcUAaG	1461	UcAUUAU A UUUUAUGA
975	gcccCUU u AgAUAgA	1463	AUAUAUU U UADGAug
987	aGaUGAU A cuUAUUG	1475	AuGgAUU c aGUAAgU
990	UGAUACU u AAuagcU	1479	AUUcaGU A AgUUAaU
1000	UGACuCU c UugCUGA	1483	aGuAAGU u AAUAUUU
1027	CgggGCU U cCUgCUC	1483	aGUAAgU U AAUAUUU
1034	UCCUGcU C CUaUcUA	1484	GUAAGUU A aUAUUUA
1037	UgcUCCU A UCUAAAU	1487	agUUAAU a UUUuUA
1039	cUccuAU c UAACUUC	1487	AgUUAAU A UUUUAUa
1039	cUCCUAU c UAACUUC	1489	UUAAUAU U uAUUAaA
1041	CcUAUcU A ACUUCaA	1489	UUAAUAU u UAUUAaA
1051	UUcAAuU U AAuAacc	1489	UUAAUAU U UAUUAaA
1148	uGAcUUU u cUuaUGU	1490	UAUAUAU u AuUAaAc
1213	GCUgGaU u UUGGAaa	1490	UAUAUAU U ADuAcAc
1213	gcUGGAU u uUgGAAA	1490	UAUAUAU U ADUacAc
1214	cugGAUU U UGGAaaA	1491	AAUAUUU a uuaCAcg
1215	ugGAUUU U GGAaaAG	1491	AAUAUUU a UuAcAcg
1234	gGGACAU c UccUUGC	1491	AaUAUUU A UuAcAcG
1236	GACAUcU c cuUGCAG	1491	AaUAUUU A UUAaAcG
1275	ugGGCCU U AcUUcUC	1494	ADuUAUU a CAcgUAU
1276	gGGCCUU A cUUcUCC	1502	cACGUaU A UaauADu
1280	CUUAcUU c UCcgUgU	1502	cAcgUAU a UAADaUU
1298	UgAAAUU a AGAaGcA	1507	AUAUAaU a UUCUaaU
1310	gcAAAGU a aAuACcA	1509	AUAUAaU U CUaAuAA
1310	GCAAAgU a aAUAcCa	1509	aUaaUaU U CUAAUAa
1310	GcaAAgU a AAUAaccA	1510	UAUAUUU C UaAuAAA
1350	AAAGCAU A AAUggU	1510	UAUAUUU C UaauAAA
1358	AAADGGU U ggGAUgU	1510	UFAUAuU c UaaUAAA
1370	UgUuaUU C AGgUAUC	1510	UzaUaUU C UAAUAAA
1375	UUCAGgU A UCAGggU	1512	aUaUUUU A AUAAAgC
1377	CAGgUAU C AGggUCA	1515	UUCUAUU A AAgCAGa
1383	UCAGggU C AcUGgAG		
1405	cccCAgU U UACUcCA		

Table 14: Human IL-6 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
86	UACACGUA AGAA GCUCCA ACCAGAGAAACACACGUGUGGURCAUUAUACUUGGUA	UGGACU GOC UACGUGUA
151	GAGUAGRA AGAA GUCOCA ACCAGAGAAACACACGUGUGGURCAUUAUACUUGGUA	UGGACU GCU UUCUACUC
172	UGGCUAUC AGAA GAGUUC ACCAGAGAAACACACGUGUGGURCAUUAUACUUGGUA	GACUCU GCU GAUAGCCA
203	UGUACAGG AGAA GGAUUC ACCAGAGAAACACACGUGUGGURCAUUAUACUUGGUA	GAUUCU GCU CCUGUACA

nt. Position	Hairpin Ribozyme Sequence	Substrate
75	AGGUGAGA AGAA GAACAC ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	GUGUUCU GAC UCUCAGCU
83	CCACACAC. AGAA GAGAGU ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	ACUUCUA GCU GUGUCUGG
147	GAGCGGAC AGAA GUGUCA ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	UGACACA GCU GUCCGCUC
150	GGUGAGGG AGAA GCUGUG ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	CACAGCU GUC CGCUCACC
154	GUCCGGUG AGAA GACAGC ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	CCUGUCC GCU CACCGAGC
168	UGGUGUUC AGAA GAGCUC ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	GGCUCU GUU GACACAGCA
199	UGAGUAGG AGAA GGRAGC ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	GUUUCCU GUC CCUCUCA
274	CCCCCAAG AGAA GUUGCA ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	UCAAACU GUC CGUGGGGG
381	AUCCAGGG AGAA GCUUG ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	CGAGCCA GUU CCUGGALU
454	CACCAUGG AGAA GUCUAG ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	CUAGGCU GCU CC AUGGUG
499	GUUUUUC AGAA GUGUAC ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	GUCACCA GAU GC AAAAAC
548	UPAAUGGA AGAA GCAUUA ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	AUAUGCU GUU UCCAUUUA
701	GCAGGAGG AGAA GAAUU ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	AAUUUCU GAU CCUCUCC
710	GARGAGGA AGAA GGAGGA ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	UCCUCCU GGC UCCUCUUC
870	AGUUCAAA AGAA GCUUG ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	CCAGGCU GAC UUGAGACU
919	CUGCGUCC AGAA GGAUUA ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	UGGUCCA GAU GGAGCCAG
1030	UGAUAUAG AGAA GGRAGC ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	GUUUCCU GCU CCUAUUA
1170	ALUGCCACA AGAA GAUUCA ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	UGAAUCA GAC UGUGCCAU
1205	CARAAUCC AGAA GUUCCA ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	UGAGCCA GCU GGAAUUUG
1402	CUGGAGUA AGAA GGGGGA ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	UCCCCCA GUU UACUCCAG
1421	ANGCAUAC AGAA GUUUUU ACCAGAGAAACACACACGUGUGUGUACAUUACUUGUA	AAAAACA GAU GUUUGCUU

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Table 16 : Mouse IL-5 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence			Substrate
75	ACUAGCA	AGAA	GAACAC	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
83	CCAGACAC	AGAA	GAAGUU	ACCAAGAAACACACACGUGUGUACAUUPACUUGUA
147	GAAGGAC	AGAA	GUGUCA	ACCAAGAAACACACACGUGUGUACAUUPACUUGUA
150	GGUAGCG	AGAA	GCUGUG	ACCAAGAAACACACACGUGUGUACAUUPACUUGUA
154	GUUGGUG	AGAA	GAACGC	ACCAAGAAACACACACGUGUGUACAUUPACUUGUA
168	UGCUUGUC	AGAA	GAAGCUC	ACCAAGAAACACACACGUGUGUACAUUPACUUGUA
199	UGAGUAGG	AGAA	GGAGGC	ACCAAGAAACACACACGUGUGUACAUUPACUUGUA
274	COOCCAGG	AGAA	GUUUUA	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
381	AUUCAGG	AGAA	GCUCUG	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
454	CACAUUGG	AGAA	GCUCAG	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
499	GUUUUUGC	AGAA	GUUGAC	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
548	UAAAUUGA	AGAA	GCUAUU	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
701	GCAGGAGG	AGAA	GAUAUU	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
710	GAAGAGGA	AGAA	GGAGGA	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
870	NEUUCANA	AGAA	GCUCUG	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
919	CUGCGUCC	AGAA	GGACCA	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
1030	UAGAUAGG	AGAA	GGAGGC	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
1170	AUGGCACA	AGAA	GAUUUA	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
1205	CANAUCC	AGAA	GCUCUA	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
1402	CUGAGUA	AGAA	GGGGGA	ACCAAGAAACACACGUGUGUACAUUPACUUGUA
1421	AAACAUAC	AGAA	GUUUUU	ACCAAGAAACACACGUGUGUACAUUPACUUGUA

Table 17

Mouse re/ A HH Target sequence

nt. Position HH Target Sequence nt. Position HH Target Sequence

19	AADGGCU a caCaGgA	467	cCAGGCU c cuguUCg
22	aGCUCcU a cGUgGOG	469	laGCCAU u AGcCAGC
26	CcUCcaU u GcGgACa	473	UuUgAGU C AGauCag
93	GAuUCGU U uCCCCUC	481	AGCGaAU C CAGAOCA
94	AuUCGUU u CCCCCA	501	AACCCCU U uCAcGUU
100	UuCCCCU C AUCUuUc	502	ACCCCCU u CAcGUUC
103	CCUCAU C UuUCCcU	508	UcCacGU U CUADAG
105	CCACUCU U uCCcUCA	509	uCAcGUU C CUADAGA
106	UCAUCU u CCcUCAG	512	cGUUCCU A UAGAgGA
129	CAGGCU C UGGgCCU	514	UGCCUuU A GAgGAGC
138	GGgCCU A UGUGGAG	534	GGGgACU A uGAcUUG
148	UGGAGAU C AUcGAaC	556	UGGcCU C UGUUCC
151	AGAUCAU c GAaCAGC	561	CCUGCU U CCAGGUG
180	AUGOGaU U CCGCUAu	562	UCUGCU C CAGGUGA
181	UGOGaU C CGCUAuA	585	aAgCCAU u AGcCAGc
186	UUCGCU A uAAaUGC	598	GGCCCCU C CuCCUGa
204	GGGCGCU C aGGGGC	613	CcCCUGU C CUcuCaC
217	GCAGuAU u CCuGGCG	616	CUGUCCU c uCaCUC
239	CACAGAU A CCACCA	617	guCCCCU C CUCAgCC
262	CCACCAU C AAGAUCA	620	CCUCCU C AgCCaug
268	UCAAGAU C AADGGCU	623	UCCUgcU u CCACUc
276	AAUGGCU A CACAGGA	628	AUCCgAU u UUUGAuA
301	UuCGaAU C UCCCUGG	630	CCgAUuU U UGAuAAc
303	CGaAUCU C CCUGGUC	631	CgAUuUU U GAuAAcC
310	CCUUGU C ACCAAGG	638	UGgCcAU u GUGuuCC
323	GGcCCU C CUcuga	661	CCGAGCU C AAGAUUC
326	uCCaCCU C ACCGGCC	667	UCAAGAU C UGCGAG
335	CCGGCCU C AuCCaCA	687	CGgAACU C UGGgAGC
349	AuGAaCU U GUgGGgA	700	GUUGCCU C GGUGGGG
352	AGaUcaU c GaAcAGc	715	AUGAGAU C UUQuUgC
375	GAUGGCU a CUADGAG	717	GAGAUUCU U CuUgCUG
376	AUGGuCU C UccGgaG	718	AGAUCUU C uUgCUGU
378	GGCUaCU A UGAGGCU	721	UuCUCCU c CaUUGcG
391	CUGAcCU C UGCCCAG	751	AaGACAU U GAGGUGU
409	GCaGuAU C CAuAGcU	759	GAGGUGU A UUUCACG
416	CCgCAGU a UCCAuAg	761	GGUGUuU U UCACGGG
417	CAuAGcU U CCAGAAC	762	GUGUuUU U CACGGGA
418	AuAGcUU C CAGAAC	763	UGUuUUU C ACGGGAC
433	UGGGgAU C CAGUGUG	792	CGAGGCU C CUUUUCU
795	GGCUCCU U UUQuCAA	1167	GAUGAGU U UuCCcCC
796	GCUCCU U UCuCAAG	1168	AUGAGUU U uCCcCCA
797	CUCCUUU U CuCAAGC	1169	UGAGUUU u CCcCCAU
798	UCCUUUU C uCAAGCU	1182	AUGcUGU U aCCaUCa
829	UGGCCAU U GUGUCC	1183	UGcUGUU a CCaUCaG

834	AUUGUGU U CCGGACu	1184	GGccccU C CUcCUGa
835	UUGUGUU C CGGACuC	1187	GUccCuU c CUcaGCc
845	GACuCCU C CgUACGC	1188	UUaCCaU C aGGGCAG
849	CCUCCgU A CGCcGAC	1198	GGgAGuU u AGuCuGa
872	cCAGGCU C CUGUuCG	1209	CAGcCCU a caCCUUC
883	UuCGaGU C UCCAUGC	1215	cuGGCCU U aGCaCCG
885	CGaGUU C CAUGCAG	1229	GGuCCU u CCucAGc
905	GCGGCU U CuGauCG	1237	CCCAgCU C CUGCCCC
906	CGGCCU C uGauCGc	1250	CCAGcCU C CAGgCuC
919	GcGAGCU C AGUGAGC	1268	CCCaGCU C CuGCCcc
936	AUGGagU U CCAGUAC	1279	CCAUUGU c cCuuCcu
937	UGGagUU C CAGUACu	1281	gUGGgCU C AGCUgcG
942	UUCCAGU A CuUGCCA	1286	ADgAGuU u UccCCCA
953	GCCuCAU c CACuAGA	1309	CuCCUGU u CgAGUCu
962	AGauGAU C GcCACCG	1315	ccccAGU u CUAaCCC
965	CagUacU u gCCaGAc	1318	CAGUuCU A aCCCCgG
973	ACCGGAU U GAaGAGA	1331	gGGuCCU C CcCAGuC
986	GAgACcU u cAGGagu	1334	CuuUuCU C AaGCUGa
996	AGGACC U A UGAGACC	1389	AGGCUU C gGAaGCC
1005	GAGACU U CAAGAGu	1413	CUGCAGU U UGADGcU
1006	AGACCU C AAGAGuA	1414	UGCAGUU U GAUGcUG
1015	AGAGuAU C AUGAAGA	1437	GGGGCU U GCUUGGC
1028	GAAGAGU C CUUCCAa	1441	CCUUGCU U GGCAACA
1031	GAGUCCU U UCAauGG	1467	GgaGUGU U CACAGAC
1032	AGUCCU U CAauGGA	1468	gaGUGUU C ACAGACC
1033	GUCCUU C AauGGAC	1482	CUGGCAU C uGUgGAC
1058	CGGCU C CAaCCCG	1486	CuUCgGU a GggAACT
1064	UaCACC U GfuCCAa	1494	GACAACT C aGAGUUU
1072	GgCGuAU U GCUUGGC	1500	UCaGAGU U UCAGCAG
1082	UGUGCU a CCGaAa	1501	CaGAGUU U CAGCAGC
1083	aaGCUU C CCGaAGu	1502	aGAGUUU C AGCAGCU
1092	CGaAaCU C AaCUUCU	1525	gGuGCAU c CCUGUGu
1097	CUCAaCU U CUGUCCC	1566	AUGGAGU A CCCUGAa
1098	UCAaCU C UGUCCC	1577	UGAaGCU A UAACTCG
1102	CUUCUGU C CCAAGC	1579	AaGCUAU A ACTCGCC
1125	CAGCCU A caCCUUC	1583	UAUAACU C GCUgGU
1127	GCCaUAU a gCcUUAC	1588	CUcUCCU A GaGAggG
1131	cAUCCU c agCaCCA	1622	CCAGCU C CUGCcCC
1132	AcaCCU c cCagCAU	1628	UCCUGCU u CggUaGG
1133	UCCaUcU c CagCuUC	1648	CGGGCU u CCAADG
1137	UUUAUu u AgCgCgc	1660	cUGaCCU C ugccCAG
1140	cCagCAU C CCUcAGC	1663	cuCUgCU U cCAGGuG
1153	GCAACAU C AACUuUG	1664	uCUgCUU c CAGGuGA
1158	ADCAACU u UGADGAG	1665	CUCgcUU u cGGAGgU
1680	GAAGACU U CUCCUCC		
1681	AAGACU C UCCUCCA		
1683	GACTUCU C CUCCADU		
1686	UUCUCCU C CAUUGCG		
1690	CCUCCAU U GCGGACA		

1704	AUGGACU U CUUAGCU
1705	UGGACUU C UCAGCUC
1707	GACUUCU C UGCUUCU
1721	uuUGAGU C AGAUCAG
1726	GUCAGAU C AGCUCCU
1731	AUCAGCU C CUAAGGU
1734	AGCUCCU A AGGUGCU
1754	CaGugCU C CCAAGAG

Table 18
Human *rel A* HH Target Sequences
nt. Position HH Target Sequence nt. Position HH Target Sequence

19	AAUGGCU C GUCUGUA	467	GCAGGCU A UCAGUCA
22	GGCUUGU C UGUAGUG	469	AGGCUAU C AGUCAGC
26	CGUCUGU A GUGCAGG	473	UAUCAGU C AGCGCAU
93	GAACUGU U CCCCCUC	481	AGCGCAU C CAGACCA
94	AACUGUU C CCCCCCA	501	AACCCCU U CCAAGUU
100	UCCCCU C ADCUCC	502	ACCCCU C CAAGUCC
103	COCUCAU C UUCOCGG	508	UCCAAGU U CCUAUAG
105	CUCACU U CCGGCA	509	CCAAGUU C CUUAAGA
106	UCAUCU C CCGGCAG	512	AGUCCU A UAGAAGA
129	CAGGCU C UGGCCCC	514	UUCUAU A GAAGAGC
138	GGCCCCU A UGUGGAG	534	GGGACU A CGACCUG
148	UGGAGAU C AUGAGAC	556	UGGGCU C UGCUUCC
151	AGAUCAU U GAGCAGC	561	CCUGCU U CCAGGUG
180	AUGGCU U CCGCUAC	562	UCUGCU C CAGGUGA
181	UGGCUU C CGCUACA	585	GACCAU C AGGCAGG
186	UUCGCU A CAAGUGC	598	GGCCCCU C CGCCUGC
204	GGGGCU C CGGGGC	613	CGCCUGU C CUUCCUC
217	GCAGCAU C CCAGGGG	616	CUUCCU U CCUCAUC
239	CACAGAU A CCACCAA	617	UGUCCU C CUCACCC
262	CCACCAU C AAGAUCA	620	CUUCCU C ADCCAU
268	UCAAGAU C AADGGCU	623	UCCUCAU C CCACCUU
276	AAUGGCU A CACAGGA	628	ADCCAU C UUUGACA
301	UGGCAU C UCCCGG	630	CCCAUCU U UGACAAU
303	CGCAUCU C CCGGUC	631	CCAUCU U GACAAUC
310	CCCGGU C ACCAAGG	638	UGACAAU C GUGCCCC
323	GGACCCU C CUCACCG	661	CCGAGCU C AAGAUUC
326	CCUCCU C ACCGGCC	667	UCAAGAU C UGCGGAG
335	CCGGCU C ACCCCCA	687	CGAAACU C UGGCAGC
349	ACGAGCU U GUAGGAA	700	GUUGCU C GUGGGGG
352	AGCUUGU A GGAAAGG	715	ADGAGAU C UUCCUAC
375	GAUGGCU U CUADGAG	717	GAGAUU U CUUACUG
376	AUGGCUU C UADGAGG	718	AGAUUU C CUACUGU
378	GGCUUCU A UGAGGCU	721	UCUCCU A CUGUGUG
391	CUGAGCU C UGCCCCG	751	AGGACAU U GAGGUGU
409	GCGCAU C CACAGUU	759	GAGGUGU A UUCACAG
416	CCACAGU U UCCAGAA	761	GGUGUAU U UCACGGG
417	CACAGUU U CCAGAAC	762	GUGUAU U CACGGGA
418	ACAGUUU C CAGAAC	763	UGUAUU C ACGGGAC
433	UGGGAU C CAGUGUG	792	CGAGGCU C CUUUUCG
795	GGCUCCU U UUGCAA	1167	GADGAGU U UCCACC
796	GCUCUUU U UCGCAAG	1168	ADGAGUU U CCAACA
797	CUCCUUU U CGCAAGC	1169	UGAGUUU C CCACCAU
798	UCCUUUU C GCAAGCU	1182	AUGGUGU U UCCUUCU
829	UGGCAU U GUGUCC	1183	UGGUGUU U CCUUCUG
834	AUGGUGU U CCGGACC	1184	GGUGUUU C CUUCUGG

835	UUGUGUU C OGGACCC	1187	GUUUCUU U CUGGGCA
845	GACCCUU C CCUACGC	1188	UUUCCUU C UGGGCAG
849	CCUCCUU A CGCAGAC	1198	GGCAGAU C AGCCAGG
872	GCAGGCU C CUGUGCG	1209	CAGGCUU C GGCCUUG
883	UGCGUGU C UCCAUGC	1215	UCCGCUU U GGCCCOG
885	CGUGUCU C CAUGCAG	1229	GGCCCUU C CCCAAGU
905	GCGGCCU U CCGACCG	1237	CCCAAGU C CUGCCCC
906	CGGCCUU C CGACCGG	1250	CCAGGCU C CAGCCOC
919	GGGAGCU C AGUGAGC	1268	CCUGCUU C CAGCCAU
936	AUGGAUU U CCAGUAC	1279	CCAUGGU A UCAGCUC
937	UGGAUUU C CAGUACC	1281	AUGGUUU C AGCCUUG
942	UUCCAGU A CCUGCCA	1286	AUCAGCU C UGGCCCA
953	GCCAGAU A CAGACGA	1309	CCCCUGU C CCAGUCC
962	AGACGAU C GUCACCG	1315	UCCAGU C CUAGUCC
965	CGAUUGU C ACOGGAU	1318	CAGUCCU A GCOCCAG
973	ACCGGAU U GAGGAGA	1331	AGGCCUU C CUCAGGC
986	GAAACGU A AAAGGAC	1334	CCCUCCU C AGCCUGU
996	AGGACAU A UGAGACC	1389	ACGCUGU C AGAGGCC
1005	CAGACCU U CAAGAGC	1413	CUGCAGU U UGAGGAU
1006	AGACCUU C AAGAGCA	1414	UGCAGUU U GAUGAUG
1015	AGAGCAU C AUGAAGA	1437	GGGGCUU U GCUUGGC
1028	GAAGAGU C CUUUCAG	1441	CCUUGCU U GGCAACA
1031	CAGUCCU U UCAGCGG	1467	GCUUGGU U CACAGAC
1032	AGUCCUU U CAGOGGA	1468	CUGUGUU C ACAGACC
1033	GUCCUUU C AGGGGAC	1482	CUGGCAU C CGUCGAC
1058	CCGGCCU C CAUCUUG	1486	CAUCCGU C GACRACU
1064	UCCACCU C GACGCAU	1494	GACRACU C CGAGUUU
1072	GACGCAU U GCUUGGC	1500	UCCGAGU U UCAGCAG
1082	UGUGCCU U CCGGCAG	1501	CCGAGUU U CAGCAGC
1083	GUGCCUU C CCGCAGC	1502	CGAGUUU C AGCAGCU
1092	CGCAGCU C AGCUUCU	1525	AGGGCAU A CCUGUGG
1097	CUCAGCU U CUGUCCC	1566	AUGGAGU A CCCUGAG
1098	UCAGCUU C UGUCCOC	1577	UGAGGCU A UAACUUG
1102	CUUCUGU C CCCAAGC	1579	AGGCUAU A ACUCGCC
1125	CAGCCCU A UCCCUUU	1583	UAUAACU C GCUUAGU
1127	GCCCUAU C CCUUUAC	1588	CCCGCCU A GUGACAG
1131	UAUCCCU U UACGUCA	1622	CCCAGCU C CUGCUCC
1132	AUCCCUU U ACGUCAU	1628	UCCUGCU C CACUGGG
1133	UCCCUUU A CGUCADC	1648	OGGGGCU C CCCA AUG
1137	UUUACGU C AUCCCUU	1660	AUGGCCU C CUUUCAG
1140	ACGUCAU C CCUGAGC	1663	GCCUCCU U UCAGGAG
1153	GCACCAU C AACUAUG	1664	CCUCCUU U CAGGAGA
1158	AUCAACU A UGAUGAG	1665	CUCCUUU C AGGAGAU
1680	GAAGACU U CUCCUCC		
1681	AAGACUU C UCCUCCA		
1683	GACUUCU C CUCCAUU		
1686	UUUCCCU C CAUUGCG		
1690	CCUCCAU U GCGGACA		
1704	ADGGACU U CUCAGCC		

1705	UGGAGUU C UCAGCCC
1707	GACUUCU C AGCCCCG
1721	GCUGAGU C AGAUCAG
1726	GUCAGAU C AGCUCCU
1731	AUCAGCU C CUAAGGG
1734	AGCUCCU A AGGGGGU
1754	CUGCCCU C CCGAGAG

Table 19
 Mouse *rel A* HH Ribozyme Sequences
 nt. HH Ribozyme Sequence
 Sequence

19	UCCUGUG	CUGAUGAGGCOGAAAGGCOGAA	AGCCAUU
22	CACCAAG	CUGAUGAGGCOGAAAGGCOGAA	AGGAGCU
26	UGUCCGC	CUGAUGAGGCOGAAAGGCOGAA	AUGGAGG
93	GAGGGGA	CUGAUGAGGCOGAAAGGCOGAA	ACAGAUU
94	UGAGGGG	CUGAUGAGGCOGAAAGGCOGAA	AACAGAU
100	GAAAGAU	CUGAUGAGGCOGAAAGGCOGAA	AGGGGAA
103	AGGGGAA	CUGAUGAGGCOGAAAGGCOGAA	AUGAGGG
105	UGAGGGA	CUGAUGAGGCOGAAAGGCOGAA	AGAUGAG
106	CUGAGGG	CUGAUGAGGCOGAAAGGCOGAA	AAGADGA
129	AGGCGCA	CUGAUGAGGCOGAAAGGCOGAA	AAGCCUG
138	CUCCACA	CUGAUGAGGCOGAAAGGCOGAA	AAGGCGC
148	GUUCGAU	CUGAUGAGGCOGAAAGGCOGAA	AUCUCCA
151	GCUGUUC	CUGAUGAGGCOGAAAGGCOGAA	AUGAUCU
180	AUAAGCG	CUGAUGAGGCOGAAAGGCOGAA	AUCGCAU
181	UAUAGCG	CUGAUGAGGCOGAAAGGCOGAA	AADCGCA
186	GCAUUAU	CUGAUGAGGCOGAAAGGCOGAA	AGCGGAA
204	GCCCGCU	CUGAUGAGGCOGAAAGGCOGAA	AGCGGCG
217	CGCCAGG	CUGAUGAGGCOGAAAGGCOGAA	AUAUCGC
239	UUGGUGG	CUGAUGAGGCOGAAAGGCOGAA	AUCUGUG
262	UGAUCUU	CUGAUGAGGCOGAAAGGCOGAA	AUGGUGG
268	AGCCAUU	CUGAUGAGGCOGAAAGGCOGAA	AUCUUGA
276	UCCUGUG	CUGAUGAGGCOGAAAGGCOGAA	AGCCAUU
301	CCAGGGA	CUGAUGAGGCOGAAAGGCOGAA	AUUCGAA
303	GACCAGG	CUGAUGAGGCOGAAAGGCOGAA	AGAUCUG
310	CCUUGGU	CUGAUGAGGCOGAAAGGCOGAA	ACCAGGG
323	UCAGGAG	CUGAUGAGGCOGAAAGGCOGAA	AGGGGCC
326	GGCCGGU	CUGAUGAGGCOGAAAGGCOGAA	AGGUGGA
335	UGUGGAU	CUGAUGAGGCOGAAAGGCOGAA	AGGCCGG
349	UCCCCAC	CUGAUGAGGCOGAAAGGCOGAA	AGUUCAU
352	GCUGUUC	CUGAUGAGGCOGAAAGGCOGAA	AUGAUCU
375	CUCAUAG	CUGAUGAGGCOGAAAGGCOGAA	AGCCAUU
376	CUCCGGA	CUGAUGAGGCOGAAAGGCOGAA	AGAUCAU
378	AGCCUCA	CUGAUGAGGCOGAAAGGCOGAA	AGUAGCC
391	CUGGGCA	CUGAUGAGGCOGAAAGGCOGAA	AGGUCAG
409	AGCUAUG	CUGAUGAGGCOGAAAGGCOGAA	AUAUCGC
416	CUAUGGA	CUGAUGAGGCOGAAAGGCOGAA	ACUGCGG
417	GUUCUGG	CUGAUGAGGCOGAAAGGCOGAA	AGCUAUG
418	GGUUCUG	CUGAUGAGGCOGAAAGGCOGAA	AAGCUAU
433	CACACUG	CUGAUGAGGCOGAAAGGCOGAA	AUCGCCA
467	OGAACAG	CUGAUGAGGCOGAAAGGCOGAA	AGCCUGG
469	GCUGGCU	CUGAUGAGGCOGAAAGGCOGAA	AUGGCUU
473	CUGAUCU	CUGAUGAGGCOGAAAGGCOGAA	ACUCAAA
481	UGGUCUG	CUGAUGAGGCOGAAAGGCOGAA	AUUCGCU

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NUC 37836

501 AACGUGA CUGAUGAGGCOGAAAGGCOGAA AGGGGUU
502 GAAAGUG CUGAUGAGGCOGAAAGGCOGAA AAGGGGU
508 CUAUAGG CUGAUGAGGCOGAAAGGCOGAA ACGUGAA
509 UCUAUAG CUGAUGAGGCOGAAAGGCOGAA AACGUGA
512 UCCUCUA CUGAUGAGGCOGAAAGGCOGAA AGGAACG
514 GCUCCUC CUGAUGAGGCOGAAAGGCOGAA AUAGGAA
534 CAAGUCA CUGAUGAGGCOGAAAGGCOGAA AGUCCCC
556 GGAAGCA CUGAUGAGGCOGAAAGGCOGAA AGGCGCA
561 CACCUUG CUGAUGAGGCOGAAAGGCOGAA AGCAGAG
562 UCACCUG CUGAUGAGGCOGAAAGGCOGAA AAGCAGA
585 GCUGGCU CUGAUGAGGCOGAAAGGCOGAA AUGGCUU
598 UCAGGAG CUGAUGAGGCOGAAAGGCOGAA AGGGGCC
613 GUGAGAG CUGAUGAGGCOGAAAGGCOGAA ACAGGGG
616 GADGUGA CUGAUGAGGCOGAAAGGCOGAA AGGACAG
617 GGCTGAG CUGAUGAGGCOGAAAGGCOGAA AAGGGAC
620 CAUGGCU CUGAUGAGGCOGAAAGGCOGAA AGGAAGG
623 GAGAUGG CUGAUGAGGCOGAAAGGCOGAA AGCAGGA
628 UADCAA CUGAUGAGGCOGAAAGGCOGAA AUCGGAU
630 GUUADCA CUGAUGAGGCOGAAAGGCOGAA AAUCCGG
631 GGUUADC CUGAUGAGGCOGAAAGGCOGAA AAAADCG
638 GGAACAC CUGAUGAGGCOGAAAGGCOGAA AUGGCCA
661 AGAUCUU CUGAUGAGGCOGAAAGGCOGAA AGCUCCG
667 CUCCGCA CUGAUGAGGCOGAAAGGCOGAA AUCUUGA
687 GCUCCCA CUGAUGAGGCOGAAAGGCOGAA AGUCCCG
700 CCCCACC CUGAUGAGGCOGAAAGGCOGAA AGGCAGC
715 GCAAGAA CUGAUGAGGCOGAAAGGCOGAA AUCCCAU
717 CAGCAAG CUGAUGAGGCOGAAAGGCOGAA AGAUCUC
718 ACAGCAA CUGAUGAGGCOGAAAGGCOGAA AAGAUCU
721 CGCAUUG CUGAUGAGGCOGAAAGGCOGAA AGGAGAA
751 ACACCC CUGAUGAGGCOGAAAGGCOGAA AUGGCUU
759 CGUGAAA CUGAUGAGGCOGAAAGGCOGAA ACACCCU
761 CCGUGA CUGAUGAGGCOGAAAGGCOGAA AUACACC
762 UCCCGUG CUGAUGAGGCOGAAAGGCOGAA AAUACAC
763 GUCCCGU CUGAUGAGGCOGAAAGGCOGAA AAUACA
792 AGAAAAG CUGAUGAGGCOGAAAGGCOGAA AGCCUCG
795 UUGAGAA CUGAUGAGGCOGAAAGGCOGAA AGGAGCC
796 CUUGAGA CUGAUGAGGCOGAAAGGCOGAA AAGGAGC
797 GCUUGAG CUGAUGAGGCOGAAAGGCOGAA AAAGGAG
798 AGCUUGA CUGAUGAGGCOGAAAGGCOGAA AAAAGGA
829 GGAACAC CUGAUGAGGCOGAAAGGCOGAA AUGGCCA
834 AGUCCCG CUGAUGAGGCOGAAAGGCOGAA ACACAAU
835 GAGUCCG CUGAUGAGGCOGAAAGGCOGAA AACACAA
845 GCGUACG CUGAUGAGGCOGAAAGGCOGAA AGGAGUC
849 GUCCCGG CUGAUGAGGCOGAAAGGCOGAA ACGGAGG
872 CGAACAG CUGAUGAGGCOGAAAGGCOGAA AGCCUGG
883 GCAUGGA CUGAUGAGGCOGAAAGGCOGAA ACUCGAA
885 CUGCAUG CUGAUGAGGCOGAAAGGCOGAA AGACUCG
905 CGAUCAG CUGAUGAGGCOGAAAGGCOGAA AGGCOGC
906 GCGADCA CUGAUGAGGCOGAAAGGCOGAA AAGGCCG

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NUC 37837

919	GCUCACU	CUGAUGAGGCOGAAAGGCOGAA	AGCUCGC
936	GUACCGG	CUGAUGAGGCOGAAAGGCOGAA	ACUCCAU
937	AGUACUG	CUGAUGAGGCOGAAAGGCOGAA	AACUCCA
942	UGGCAAG	CUGAUGAGGCOGAAAGGCOGAA	ACUGGAA
953	UCAUGUG	CUGAUGAGGCOGAAAGGCOGAA	AUGAGGC
962	OGGUGGC	CUGAUGAGGCOGAAAGGCOGAA	ADCAUCU
965	GUCUGGC	CUGAUGAGGCOGAAAGGCOGAA	AGUACUG
973	UCUCUUC	CUGAUGAGGCOGAAAGGCOGAA	ADCCGGU
986	ACUCUUG	CUGAUGAGGCOGAAAGGCOGAA	AGGUCUC
996	GGUCUCA	CUGAUGAGGCOGAAAGGCOGAA	AGGUCCU
1005	ACUCUUG	CUGAUGAGGCOGAAAGGCOGAA	AGGUCUC
1006	UACUCUU	CUGAUGAGGCOGAAAGGCOGAA	AAGGUCU
1015	UCUUCAU	CUGAUGAGGCOGAAAGGCOGAA	AUACUCU
1028	UUGAAAG	CUGAUGAGGCOGAAAGGCOGAA	ACUCUUC
1031	CCAUUGA	CUGAUGAGGCOGAAAGGCOGAA	AGGACUC
1032	UCCAUUG	CUGAUGAGGCOGAAAGGCOGAA	AAGGACU
1033	GUCCAUU	CUGAUGAGGCOGAAAGGCOGAA	AAAGGAC
1058	OGGGUUG	CUGAUGAGGCOGAAAGGCOGAA	AGGCCGG
1064	UUGGAUC	CUGAUGAGGCOGAAAGGCOGAA	AGGUGUA
1072	GCACAGC	CUGAUGAGGCOGAAAGGCOGAA	AUACGCC
1082	UUUCGGG	CUGAUGAGGCOGAAAGGCOGAA	AGGCACA
1083	ACUUCGG	CUGAUGAGGCOGAAAGGCOGAA	AAGGCUU
1092	AGAAGUU	CUGAUGAGGCOGAAAGGCOGAA	AGUUUUG
1097	GGGACAG	CUGAUGAGGCOGAAAGGCOGAA	AGUUGAG
1098	GGGGACA	CUGAUGAGGCOGAAAGGCOGAA	AAGUUGA
1102	GCUUGGG	CUGAUGAGGCOGAAAGGCOGAA	ACAGUAG
1125	GAAGGUG	CUGAUGAGGCOGAAAGGCOGAA	AGGGCUG
1127	GUAGGC	CUGAUGAGGCOGAAAGGCOGAA	AUAGGCG
1131	UGGUGCU	CUGAUGAGGCOGAAAGGCOGAA	AGGGAUD
1132	AUGCUGG	CUGAUGAGGCOGAAAGGCOGAA	AAGGUGU
1133	GAAGCUG	CUGAUGAGGCOGAAAGGCOGAA	AGAUGGA
1137	GCGGCUU	CUGAUGAGGCOGAAAGGCOGAA	AAGUAAA
1140	GCUGAGG	CUGAUGAGGCOGAAAGGCOGAA	AUGCUGG
1153	CAAAGUU	CUGAUGAGGCOGAAAGGCOGAA	AUGGUGC
1158	CUCAUCA	CUGAUGAGGCOGAAAGGCOGAA	AGUUGAU
1167	GGGGGAA	CUGAUGAGGCOGAAAGGCOGAA	ACUCAUC
1168	UGGGGGA	CUGAUGAGGCOGAAAGGCOGAA	AACUCAU
1169	AUGGGGG	CUGAUGAGGCOGAAAGGCOGAA	AAACUCA
1182	UGADGGU	CUGAUGAGGCOGAAAGGCOGAA	ACAGCAU
1183	CUGAUGG	CUGAUGAGGCOGAAAGGCOGAA	AACAGCA
1184	UCAGGAG	CUGAUGAGGCOGAAAGGCOGAA	AGGGGCC
1187	GGCUGAG	CUGAUGAGGCOGAAAGGCOGAA	AAGGGAC
1188	CUGCCCU	CUGAUGAGGCOGAAAGGCOGAA	AUGGUAA
1198	UCAGACU	CUGAUGAGGCOGAAAGGCOGAA	AACUCCC
1209	GAAGGUG	CUGAUGAGGCOGAAAGGCOGAA	AGGGCUG
1215	OGGUGCU	CUGAUGAGGCOGAAAGGCOGAA	AGGOCAG
1229	GCUGAGG	CUGAUGAGGCOGAAAGGCOGAA	AGGGACC
1237	GGGGCAG	CUGAUGAGGCOGAAAGGCOGAA	AGCUGGG
1250	GAGCCUG	CUGAUGAGGCOGAAAGGCOGAA	AGGCUGG

1268	GGGGCAG	CUGADGAGGCOGAAAGGCOGAA	AGCUGGG
1279	AGGAAGG	CUGADGAGGCOGAAAGGCOGAA	ACCAUGG
1281	CGCAGCU	CUGADGAGGCOGAAAGGCOGAA	AGCCAC
1286	UGGGGA	CUGADGAGGCOGAAAGGCOGAA	AACUCAU
1309	AGACUCG	CUGADGAGGCOGAAAGGCOGAA	ACAGGAG
1315	GGGUAG	CUGADGAGGCOGAAAGGCOGAA	ACUGGG
1318	CCGGGU	CUGADGAGGCOGAAAGGCOGAA	AGAACUG
1331	GACUGG	CUGADGAGGCOGAAAGGCOGAA	AGGACC
1334	UCAGCUU	CUGADGAGGCOGAAAGGCOGAA	AGAAAAG
1389	GGUUC	CUGADGAGGCOGAAAGGCOGAA	ACAGCGU
1413	AGCAUCA	CUGADGAGGCOGAAAGGCOGAA	ACUGCAG
1414	CAGCAUC	CUGADGAGGCOGAAAGGCOGAA	AACUGCA
1437	GCCAGC	CUGADGAGGCOGAAAGGCOGAA	AGGCCC
1441	UGUUGCC	CUGADGAGGCOGAAAGGCOGAA	AGCAAGG
1467	GUCUGUG	CUGADGAGGCOGAAAGGCOGAA	ACACUCC
1468	GGUCUGU	CUGADGAGGCOGAAAGGCOGAA	AACACUC
1482	GUCCACA	CUGADGAGGCOGAAAGGCOGAA	ADGCCAG
1486	AGUUCCC	CUGADGAGGCOGAAAGGCOGAA	ACCGAAG
1494	AAACUCU	CUGADGAGGCOGAAAGGCOGAA	AGUUGUC
1500	CUGCUGA	CUGADGAGGCOGAAAGGCOGAA	ACUCUGA
1501	GCUGCUG	CUGADGAGGCOGAAAGGCOGAA	AACUCUG
1502	AGCUGCU	CUGADGAGGCOGAAAGGCOGAA	AAACUCU
1525	ACACAGG	CUGADGAGGCOGAAAGGCOGAA	ADGCCAC
1566	UUCAGGG	CUGADGAGGCOGAAAGGCOGAA	ACUCCAU
1577	CGAGUUA	CUGADGAGGCOGAAAGGCOGAA	AGCUUCA
1579	GGGAGU	CUGADGAGGCOGAAAGGCOGAA	ADAGCUU
1583	ACCAGGC	CUGADGAGGCOGAAAGGCOGAA	AGUUAUA
1588	CCUCUC	CUGADGAGGCOGAAAGGCOGAA	AGGAGAG
1622	GGGGCAG	CUGADGAGGCOGAAAGGCOGAA	AGCUGGG
1628	CCUACCG	CUGADGAGGCOGAAAGGCOGAA	AGCAGGA
1648	CAUUGGG	CUGADGAGGCOGAAAGGCOGAA	AGCCCCG
1660	CDGGGCA	CUGADGAGGCOGAAAGGCOGAA	AGGUCAG
1663	CACCUGG	CUGADGAGGCOGAAAGGCOGAA	AGCAGAG
1664	UCACCUG	CUGADGAGGCOGAAAGGCOGAA	AAGCAGA
1665	ACCUCOG	CUGADGAGGCOGAAAGGCOGAA	AAGCGAG
1680	GGAGGAG	CUGADGAGGCOGAAAGGCOGAA	AGUCUUC
1681	UGGAGGA	CUGADGAGGCOGAAAGGCOGAA	AAGUCUU
1683	AADGGAG	CUGADGAGGCOGAAAGGCOGAA	AGAAGUC
1686	CGCAUUG	CUGADGAGGCOGAAAGGCOGAA	AGGACAA
1690	UGUCCGC	CUGADGAGGCOGAAAGGCOGAA	ADGGAGG
1704	AGCAGAG	CUGADGAGGCOGAAAGGCOGAA	AGUCCAU
1705	GAGCAGA	CUGADGAGGCOGAAAGGCOGAA	AAGUCCA
1707	AAGAGCA	CUGADGAGGCOGAAAGGCOGAA	AGAAGUC
1721	CUGAUCU	CUGADGAGGCOGAAAGGCOGAA	ACUCAAA
1726	AGGAGCU	CUGADGAGGCOGAAAGGCOGAA	ADUCGAC
1731	ACCUUAG	CUGADGAGGCOGAAAGGCOGAA	AGCUGAU
1734	AGCACCU	CUGADGAGGCOGAAAGGCOGAA	AGGAGCU
1754	CUCUUGG	CUGADGAGGCOGAAAGGCOGAA	AGCACUG

Table 20
Human *rel A* HH Ribozyme Sequences
nt. Position HH Ribozyme Sequences

19	UACAGAC	CUGAUGAGGCOGAAAGGCOGAA	AGCCAUU
22	CACUACA	CUGAUGAGGCOGAAAGGCOGAA	ACGAGCC
26	CGUGCAC	CUGAUGAGGCOGAAAGGCOGAA	ACAGACG
93	GAGGGGG	CUGAUGAGGCOGAAAGGCOGAA	ACAGUUC
94	UGAGGGG	CUGAUGAGGCOGAAAGGCOGAA	AACAGUU
100	GGAAGAU	CUGAUGAGGCOGAAAGGCOGAA	AGGGGGA
103	CCGGGAA	CUGAUGAGGCOGAAAGGCOGAA	ADGAGGG
105	UGCOGGG	CUGAUGAGGCOGAAAGGCOGAA	AGAUGAG
106	CUGCOGG	CUGAUGAGGCOGAAAGGCOGAA	AAGADGA
129	GGGGCCA	CUGAUGAGGCOGAAAGGCOGAA	AGGCCUG
138	CUCCACA	CUGAUGAGGCOGAAAGGCOGAA	AGGGGCC
148	GTUCAAU	CUGAUGAGGCOGAAAGGCOGAA	AUCUCCA
151	GCUGCUC	CUGAUGAGGCOGAAAGGCOGAA	ADGADCU
180	GUAGCGG	CUGAUGAGGCOGAAAGGCOGAA	AGGCCAU
181	UGUAGGG	CUGAUGAGGCOGAAAGGCOGAA	AAGGCCA
186	GCACUUG	CUGAUGAGGCOGAAAGGCOGAA	AGCGGAA
204	GCCCGCG	CUGAUGAGGCOGAAAGGCOGAA	AGCGGCC
217	CGCCUGG	CUGAUGAGGCOGAAAGGCOGAA	AUGCUGC
239	UUGGUGG	CUGAUGAGGCOGAAAGGCOGAA	AUCUGUG
262	UGAUCUU	CUGAUGAGGCOGAAAGGCOGAA	ADGGUGG
268	AGCCAUU	CUGAUGAGGCOGAAAGGCOGAA	AUCUUGA
276	UCCUGUG	CUGAUGAGGCOGAAAGGCOGAA	AGCCAUU
301	CCAGGGA	CUGAUGAGGCOGAAAGGCOGAA	AUGGCCA
303	GACCAAG	CUGAUGAGGCOGAAAGGCOGAA	AGAUGCG
310	CCUUGGU	CUGAUGAGGCOGAAAGGCOGAA	ACCAGGG
323	CGGUGAG	CUGAUGAGGCOGAAAGGCOGAA	AGGGUCC
326	GGCOGGU	CUGAUGAGGCOGAAAGGCOGAA	AGGAGGG
335	UGGGGGU	CUGAUGAGGCOGAAAGGCOGAA	AGGCCGG
349	UUCCUAC	CUGAUGAGGCOGAAAGGCOGAA	AGCUUGU
352	CCUUUCC	CUGAUGAGGCOGAAAGGCOGAA	ACAAGCU
375	CUCAUAG	CUGAUGAGGCOGAAAGGCOGAA	AGCCAUU
376	CCUCANA	CUGAUGAGGCOGAAAGGCOGAA	AAGCCAU
378	AGCCUCA	CUGAUGAGGCOGAAAGGCOGAA	AGAAGCC
391	CCGGGCA	CUGAUGAGGCOGAAAGGCOGAA	AGCUCAG
409	AACUGUG	CUGAUGAGGCOGAAAGGCOGAA	AUGCAGC
416	UUCUGGA	CUGAUGAGGCOGAAAGGCOGAA	ACUGUGG
417	GUUCUGG	CUGAUGAGGCOGAAAGGCOGAA	AACUGUG
418	GGUUCUG	CUGAUGAGGCOGAAAGGCOGAA	AAACUGU
433	CACACUG	CUGAUGAGGCOGAAAGGCOGAA	AUUCCCA
467	UGACUGA	CUGAUGAGGCOGAAAGGCOGAA	AGCCUUC
469	GCUGACU	CUGAUGAGGCOGAAAGGCOGAA	AUAGCCU
473	AUGGCGU	CUGAUGAGGCOGAAAGGCOGAA	ACUGAUA
481	UGGUCUG	CUGAUGAGGCOGAAAGGCOGAA	AUGGCGU
501	AACUUGG	CUGAUGAGGCOGAAAGGCOGAA	AGGGGUU

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NUC 37840

502	GAACUUG	CUGAUGAGGCOOGAAAGGCOOGAA	AAGGGGU
508	CUAIDAG	CUGAUGAGGCOOGAAAGGCOOGAA	ACUUGGA
509	UCIAIAG	CUGAUGAGGCOOGAAAGGCOOGAA	AACUUGG
512	UCUUCIA	CUGAUGAGGCOOGAAAGGCOOGAA	AGGAACU
514	GCUCUUC	CUGAUGAGGCOOGAAAGGCOOGAA	AUAGGAA
534	CAGGUCG	CUGAUGAGGCOOGAAAGGCOOGAA	AGUCCCC
556	GGAAGCA	CUGAUGAGGCOOGAAAGGCOOGAA	AGCCGCA
561	CACCUUG	CUGAUGAGGCOOGAAAGGCOOGAA	AGCAGAG
562	UCACCUG	CUGAUGAGGCOOGAAAGGCOOGAA	AAGCAGA
585	CCUGCCU	CUGAUGAGGCOOGAAAGGCOOGAA	AUGGGUC
598	GCAAGCG	CUGAUGAGGCOOGAAAGGCOOGAA	AGGGGCC
613	GAGGAAG	CUGAUGAGGCOOGAAAGGCOOGAA	ACAGGCG
616	GAUGAGG	CUGAUGAGGCOOGAAAGGCOOGAA	AGGACAG
617	GGADGAG	CUGAUGAGGCOOGAAAGGCOOGAA	AAGGACA
620	AUGGGAU	CUGAUGAGGCOOGAAAGGCOOGAA	AGGAAGG
623	AAGADGG	CUGAUGAGGCOOGAAAGGCOOGAA	AUGAGGA
628	UGUCAA	CUGAUGAGGCOOGAAAGGCOOGAA	AUGGGAU
630	ADUGUCA	CUGAUGAGGCOOGAAAGGCOOGAA	AGADGGG
631	GADUGUC	CUGAUGAGGCOOGAAAGGCOOGAA	AAGAUGG
638	GGGGCAC	CUGAUGAGGCOOGAAAGGCOOGAA	ADUGUCA
661	AGADCUU	CUGAUGAGGCOOGAAAGGCOOGAA	AGCUUGG
667	CUUGGCA	CUGAUGAGGCOOGAAAGGCOOGAA	ADCUUGA
687	GCUGCCA	CUGAUGAGGCOOGAAAGGCOOGAA	AGUUUUG
700	CCCCACC	CUGAUGAGGCOOGAAAGGCOOGAA	AGGCAGC
715	GUAGGAA	CUGAUGAGGCOOGAAAGGCOOGAA	AUCUCAU
717	CAGUAGG	CUGAUGAGGCOOGAAAGGCOOGAA	AGADUUC
718	ACAGUAG	CUGAUGAGGCOOGAAAGGCOOGAA	AAGADCU
721	CACACAG	CUGAUGAGGCOOGAAAGGCOOGAA	AGGAAGA
751	ACACCTC	CUGAUGAGGCOOGAAAGGCOOGAA	AUGUCCU
759	CGUGAAA	CUGAUGAGGCOOGAAAGGCOOGAA	ACACCTC
761	CCCGUGA	CUGAUGAGGCOOGAAAGGCOOGAA	ATAACCC
762	UCCCGUG	CUGAUGAGGCOOGAAAGGCOOGAA	AAUACAC
763	GUCCCGU	CUGAUGAGGCOOGAAAGGCOOGAA	AAAUACA
792	CGAAAAG	CUGAUGAGGCOOGAAAGGCOOGAA	AGCCUUG
795	UUGCGAA	CUGAUGAGGCOOGAAAGGCOOGAA	AGGAGGC
796	CUUGCGA	CUGAUGAGGCOOGAAAGGCOOGAA	AAGGAGC
797	GCUUGCG	CUGAUGAGGCOOGAAAGGCOOGAA	AAAGGAG
798	AGCUUGC	CUGAUGAGGCOOGAAAGGCOOGAA	AAAAGGA
829	GGAACAC	CUGAUGAGGCOOGAAAGGCOOGAA	AUGGCCA
834	GGUCCGG	CUGAUGAGGCOOGAAAGGCOOGAA	ACACAAU
835	GGGUCCG	CUGAUGAGGCOOGAAAGGCOOGAA	AACACAA
845	GCGUAGG	CUGAUGAGGCOOGAAAGGCOOGAA	AGGGGUC
849	GUCUGCG	CUGAUGAGGCOOGAAAGGCOOGAA	AGGGAGG
872	CGCACAG	CUGAUGAGGCOOGAAAGGCOOGAA	AGCCUUG
883	GCAUGGA	CUGAUGAGGCOOGAAAGGCOOGAA	ACACGCA
885	CUGCAGG	CUGAUGAGGCOOGAAAGGCOOGAA	AGACACG
905	CGGUCCG	CUGAUGAGGCOOGAAAGGCOOGAA	AGGCOGC
906	CCGGUUG	CUGAUGAGGCOOGAAAGGCOOGAA	AAGGCOG
919	GUUACAU	CUGAUGAGGCOOGAAAGGCOOGAA	AGCUCCC

936	GUACUGG	CUGAUGAGGCGAAAGGCGGAA	AUUCCAU
937	GGUACUG	CUGAUGAGGCGGAAAGGCGGAA	AAUUGCA
942	UGGCAGG	CUGAUGAGGCGGAAAGGCGGAA	ACUGGAA
953	UCGUCUG	CUGAUGAGGCGGAAAGGCGGAA	AUCUGGC
962	CGGUGAC	CUGAUGAGGCGGAAAGGCGGAA	AUGGUCU
965	AUCCGGU	CUGAUGAGGCGGAAAGGCGGAA	ACGADUG
973	UCUCCUC	CUGAUGAGGCGGAAAGGCGGAA	AUCCGGU
986	GUCCUUU	CUGAUGAGGCGGAAAGGCGGAA	ACGUUUC
996	GGUCUCA	CUGAUGAGGCGGAAAGGCGGAA	AUGUCCU
1005	GCUCUUG	CUGAUGAGGCGGAAAGGCGGAA	AGGUCUC
1006	UGCUCUU	CUGAUGAGGCGGAAAGGCGGAA	AAGGUCU
1015	UCUUCAU	CUGAUGAGGCGGAAAGGCGGAA	AUGGUCU
1028	CUGAAAG	CUGAUGAGGCGGAAAGGCGGAA	ACUCUUC
1031	CCGCUGA	CUGAUGAGGCGGAAAGGCGGAA	AGGACUC
1032	UCGCGUG	CUGAUGAGGCGGAAAGGCGGAA	AAGGACU
1033	GUCCGCU	CUGAUGAGGCGGAAAGGCGGAA	AAAGGAC
1058	CGAGGUG	CUGAUGAGGCGGAAAGGCGGAA	AGGCGG
1064	ADGCGUC	CUGAUGAGGCGGAAAGGCGGAA	AGGUGGA
1072	GCACAGC	CUGAUGAGGCGGAAAGGCGGAA	AUGGUCU
1082	CUGCGGG	CUGAUGAGGCGGAAAGGCGGAA	AGGACAC
1083	GCUGGGG	CUGAUGAGGCGGAAAGGCGGAA	AAAGGAC
1092	AGAAGCU	CUGAUGAGGCGGAAAGGCGGAA	AGCUGCG
1097	GGGACAG	CUGAUGAGGCGGAAAGGCGGAA	AGCUGAG
1098	GGGACA	CUGAUGAGGCGGAAAGGCGGAA	AAAGCUA
1102	GCUUGGG	CUGAUGAGGCGGAAAGGCGGAA	ACAGGAG
1125	AAAGGGA	CUGAUGAGGCGGAAAGGCGGAA	AGGCGUG
1127	GUAAAGG	CUGAUGAGGCGGAAAGGCGGAA	AUAGGCG
1131	UGACGUA	CUGAUGAGGCGGAAAGGCGGAA	AGGGAUA
1132	AUGACGU	CUGAUGAGGCGGAAAGGCGGAA	AAAGGCU
1133	GAUGACG	CUGAUGAGGCGGAAAGGCGGAA	AAAGGGA
1137	CAGGGAU	CUGAUGAGGCGGAAAGGCGGAA	ACGUAAA
1140	GCUCAGG	CUGAUGAGGCGGAAAGGCGGAA	AUGACGU
1153	CAUAGUU	CUGAUGAGGCGGAAAGGCGGAA	AUGGUGC
1158	CUCAUCA	CUGAUGAGGCGGAAAGGCGGAA	AGUUGAU
1167	GGUGGGA	CUGAUGAGGCGGAAAGGCGGAA	ACUCAUC
1168	UGGUGGG	CUGAUGAGGCGGAAAGGCGGAA	AAUCUUA
1169	AUGGUGG	CUGAUGAGGCGGAAAGGCGGAA	AAACUCA
1182	AGAAGGA	CUGAUGAGGCGGAAAGGCGGAA	ACACCAU
1183	CAGGAGG	CUGAUGAGGCGGAAAGGCGGAA	AAACCCA
1184	CCAGAAG	CUGAUGAGGCGGAAAGGCGGAA	AAACACC
1187	UGCCAG	CUGAUGAGGCGGAAAGGCGGAA	AGGAAAC
1188	CUGCCCA	CUGAUGAGGCGGAAAGGCGGAA	AAGGAAA
1198	CCUGGCU	CUGAUGAGGCGGAAAGGCGGAA	AUCUGCC
1209	CAAGGCC	CUGAUGAGGCGGAAAGGCGGAA	AGGOCUG
1215	CGGGGCC	CUGAUGAGGCGGAAAGGCGGAA	AGGCGGA
1229	ACUUGGG	CUGAUGAGGCGGAAAGGCGGAA	AGGGGCC
1237	GGGGCAG	CUGAUGAGGCGGAAAGGCGGAA	ACUUGGG
1250	GGGGCUG	CUGAUGAGGCGGAAAGGCGGAA	AGCCUGG
1268	AUGGCGU	CUGAUGAGGCGGAAAGGCGGAA	AGCAGGG

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NUC 37842

1279	GAGCUGA	CUGAUGAGGCOGAAAGGCOGAA	ACCAUGG
1281	CAGAGCU	CUGAUGAGGCOGAAAGGCOGAA	AUAACAU
1286	UGGGCCA	CUGAUGAGGCOGAAAGGCOGAA	AGCUGAU
1309	GGACUGG	CUGAUGAGGCOGAAAGGCOGAA	ACAGGGG
1315	GGGCUAG	CUGAUGAGGCOGAAAGGCOGAA	ACUGGGA
1318	CUGGGGC	CUGAUGAGGCOGAAAGGCOGAA	AGGACUG
1331	GCCUGAG	CUGAUGAGGCOGAAAGGCOGAA	AGGGCCU
1334	ACAGCCU	CUGAUGAGGCOGAAAGGCOGAA	AGGAGGG
1389	GGCCUCU	CUGAUGAGGCOGAAAGGCOGAA	ACAGCGU
1413	AUCAUCA	CUGAUGAGGCOGAAAGGCOGAA	ACUGCAG
1414	CAUCAUC	CUGAUGAGGCOGAAAGGCOGAA	AACUGCA
1437	GCCAAGC	CUGAUGAGGCOGAAAGGCOGAA	AGGCCCC
1441	UGUUGOC	CUGAUGAGGCOGAAAGGCOGAA	AGCAAGG
1467	GUCUGUG	CUGAUGAGGCOGAAAGGCOGAA	ACACAGC
1468	GGCCUGU	CUGAUGAGGCOGAAAGGCOGAA	AACACAG
1482	GUOGACG	CUGAUGAGGCOGAAAGGCOGAA	AUGCCAG
1486	AGUUGUC	CUGAUGAGGCOGAAAGGCOGAA	ACGGAUG
1494	AAACUCG	CUGAUGAGGCOGAAAGGCOGAA	AGUUGUC
1500	CUGCUGA	CUGAUGAGGCOGAAAGGCOGAA	ACUCCGA
1501	GCUGCUG	CUGAUGAGGCOGAAAGGCOGAA	AAUCCGG
1502	AGCUGCU	CUGAUGAGGCOGAAAGGCOGAA	AAACUCG
1525	CCACAGG	CUGAUGAGGCOGAAAGGCOGAA	AUGCCCU
1566	CUCAGGG	CUGAUGAGGCOGAAAGGCOGAA	ACUCCAU
1577	CGAGUUA	CUGAUGAGGCOGAAAGGCOGAA	AGCCUCA
1579	GGCGAGU	CUGAUGAGGCOGAAAGGCOGAA	AUAACCU
1583	ACUAGGC	CUGAUGAGGCOGAAAGGCOGAA	AGUUAUA
1588	CUGUCAC	CUGAUGAGGCOGAAAGGCOGAA	AGGCGAG
1622	GGAGCAG	CUGAUGAGGCOGAAAGGCOGAA	AGCUGGG
1628	CCACUGG	CUGAUGAGGCOGAAAGGCOGAA	AGCAGGA
1648	CAUUGGG	CUGAUGAGGCOGAAAGGCOGAA	AGCCCCG
1660	CUGAAAG	CUGAUGAGGCOGAAAGGCOGAA	AGGCCAU
1663	CUCCUGA	CUGAUGAGGCOGAAAGGCOGAA	AGGAGGC
1664	UCUCCUG	CUGAUGAGGCOGAAAGGCOGAA	AAGGAGG
1665	AUCUCCU	CUGAUGAGGCOGAAAGGCOGAA	AAAGGAG
1680	GGAGGAG	CUGAUGAGGCOGAAAGGCOGAA	AGUCUUC
1681	UGGAGGA	CUGAUGAGGCOGAAAGGCOGAA	AAGUCUU
1683	AAUGGAG	CUGAUGAGGCOGAAAGGCOGAA	AGRAGUC
1686	CGCAUUG	CUGAUGAGGCOGAAAGGCOGAA	AGGAGAA
1690	UGUCCGC	CUGAUGAGGCOGAAAGGCOGAA	AUGGAGG
1704	GGCUGAG	CUGAUGAGGCOGAAAGGCOGAA	AGUCCAU
1705	GGGCUCA	CUGAUGAGGCOGAAAGGCOGAA	AAGUCCA
1707	CAGGGCU	CUGAUGAGGCOGAAAGGCOGAA	AGAAGUC
1721	CUGAUCU	CUGAUGAGGCOGAAAGGCOGAA	ACUCAGC
1726	AGGAGCU	CUGAUGAGGCOGAAAGGCOGAA	AUCUGAC
1731	CCCUUAG	CUGAUGAGGCOGAAAGGCOGAA	AGCUGAU
1734	ACCCCCU	CUGAUGAGGCOGAAAGGCOGAA	AGGAGCU
1754	CUCUGGG	CUGAUGAGGCOGAAAGGCOGAA	AGGGCAG

Table 21
Human re/ A Hairpin Ribozyme/Target Sequences
nt. Position Hairpin Ribozyme sequence Substrate

90	UGAGGGGG AGAA GUUC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GAACU GUU CCCCCUCA
156	GCUGCUUG AGAA GTUC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GAGCA GCC CAAGCAGC
362	GCCAUCCTC AGAA GUCC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GAACU GCC GCGAUGGC
413	GUUCUGGA AGAA GUGG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CCACA GUU UCCAGAAC
606	GAAGGACA AGAA GCAG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CUGCC GCC UGUCCUUC
652	UUGAGCTUC AGAA GUGU ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	ACACU GCC GAGCTUCA
695	CCCACCCGA AGAA GTUG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CAGCU GCC UCGGUGGG
853	AGGCTUGGG AGAA GCGU ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	ACCCA GAC CCCAGCCU
900	GGUCGGAA AGAA GCCG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CGGCG GCC UUCGGACC
955	UGACGAUC AGAA GUUU ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AUACA GAC GAUCGUCA
1037	GUCCGUGG AGAA GTUG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CAGCG GAC CCACCCAC
1045	GGCCGGGG AGAA GUGG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CCACC GAC CCCCAGCC
1410	CAUCAUCA AGAA GCAG ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CUCCA GUU UGAUGAUG
1453	ACAGCTUG AGAA GUGC ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GCACA GAC CCAGCTGU
1471	GAUGCCAG AGAA GUGA ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UCACA GAC CUGGCAUC

Table 22
Mouse *relA* Hairpin Ribozyme/Target Sequences
nt. Position Hairpin Ribozyme sequence Substrate

137	GUUGCTUC AGAA GUUC ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	GAACA GCC GAAGCAAC
273	GAGAUUCG AGAA GUUC ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	GAACA GUU CGAAUUCUC
343	GCCAUCCC AGAA GUCC ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	GGACU GCC GGGAUUGGC
366	GGGACAGAG AGAA GCTU ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	AGGCU GAC CUCUGGCC
633	UUGAGCTUC AGAA GUGU ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	ACACU GCC GAGCUCAA
676	CCCACCGA AGAA GTUC ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	GAGCU GCC UCGUGGGG
834	AGGCTUGG AGAA GCGU ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	ACGCC GAC CCCAGCCTU
881	GAUCAGAA AGAA GCGG ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	CGCGG GCC UUCUGAUC
1100	AGGUGUAG AGAA GCGG ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	CGGCA GCC CUACACCU
1205	GGGCAGAG AGAA GUGC ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	GCACC GUC CUCUGCCC
1361	GGGCTUCC AGAA GCGU ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	AGGCU GUC GGAAGCCC
1385	CAGCAUCA AGAA GCAG ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	CUGCA GUU UGAUGCTG
1431	ACUGCTUG AGAA GUUC ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	GCACA GAC CCAGGAGU
1449	GAUGCCAG AGAA GUGA ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	UCACA GAC CUGGCAUC
1802	AAGUGGGG AGAA GCTG ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	CAGCU GCC CCCGACUU
2009	UGGCTUCC AGAA GUCC ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	GGACA GAC UGGAGCCA
2124	UGGUGUCC AGAA GCAC ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	GUCCU GCC CGACACCA
2233	AUUCUGAA AGAA GCCA ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	UGGCC GCC UUCAGAAU
2354	UCAGUAAA AGAA GUCU ACCAGAGAAACACACGUGUGGUACAUUACCTUGGUA	AGACA GCC UUUACUGA

Table 23: Human TNF- α HH Ribozyme Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
28	GGCAGGU U CUCUCC	321	GUCAGAU C AUCUUCU
29	GCAGGUU C UCUUCCU	324	AGAUCAU C UUCUGA
31	AGGUUCU C UUCUCU	326	AUCAUCU U CUCGAAC
33	GUUCUCU U CUCUCA	327	UCAUCU C UCGAAC
34	UUCUCU C CUCUCAC	329	AUCUUCU C GAACCC
37	UCUUCU C UCACUA	352	AGCCUGU A GCCCAG
39	UUCUCU C ACAUACU	361	CCCAUGU U GUAGCA
44	CUCACAU A CUGACCC	364	AUGUGU A GCAAAC
58	CACGGCU C CACCCU	374	AAACCCU C AAGCUA
65	CCACCCU C UCUCCC	391	GGCAGCU C CAGUGG
67	ACCCUCU C UCCCUU	421	AUGCCCU C CUGGCA
69	CCUCUCU C CUCUGA	449	GAGAGAU A ACCAGCU
106	GCAUGAU C CGGGACG	468	GUGOCAU C AGAGGG
136	AGGGCU C CCAAGX	480	GGCCUGU A CCUCAC
165	CAGGGCU C CAGGGG	484	UGUACU C AUCUACU
177	CGGUGCU U GUUCUC	487	ACUCAU C UACUCC
180	UGCUUGU U CUCAGC	489	CUCAUCU A CUCCCAG
181	GCUUGU C CUCAGC	492	AUCUACU C CCAGGUC
184	UGUUCU C AGCCUCU	499	CCAGGU C CUCUCA
190	UCAGCCU C UUCUCU	502	AGGUCCU C UUCAAGG
192	AGCCUCU U CUCUUC	504	GUCUCU U CAAGGG
193	GCCUCU C UCUUCC	505	UCCUCU C AAGGGC
195	CUCUCU C CUCUCU	525	UGCCCU C CACCAU
198	UUCUCU U CUCGAC	538	AUGUGCU C CUCACC
199	UCUCUCU C CUGAUG	541	UGUCCU C ACCACA
205	UCUGAU C GUGGCAG	553	ACACCAU C AGCCCA
226	CCAGCU C UUCUGC	562	GGGCAU C GCGUCU
228	AGGCUU U CUGCUG	568	UGGCGU C UCCUAC
229	CGCUCU C UGCGUC	570	GCGUCU C CUAACAG
243	CUGCACU U UGGAGU	573	GUCUCU A CCAGAC
244	UGCACU U GGAGUA	586	CCAAGU C AACCCU
253	GAGUGAU C GGCCCC	592	UCAACU C CUCUCU
273	GAAGAU C CCCCAG	595	ACUCCU C UCUGCA
286	GGGACU C UCUCUA	597	CUCUCU C UGCAUC
288	GACUCU C UCUAUC	604	CUGOCAU C AAGAGC
290	CCUCUCU C UAADCAG	657	CCUGGU A UGAGCC
292	UCUCUCU A AUCAGC	667	AGCCAU C UAUUGG
295	CCUAAU C AGCCUC	669	CCCAUCU A UCUGGA
302	CAGCCU C UGGCCA		

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671	CAUCUAT C UGGGAGG	960	UGGGAUU C AGGA AUG
682	GAGGGGU C UUCAGC	1001	AACCACU A AGAUUC
684	GGGGUCU U CCAGCUG	1007	UAAGAAU U CAAACUG
685	GGGUCUU C CAGCUGG	1008	AAGAAUU C AAACUGG
709	ACCGACU C AGGCGUG	1021	GGGGCCU C CAGAACU
721	CUGAGAU C AAUCGGC	1029	CAGAACU C ACUGGGG
725	GAUCAAU C GGCCCGA	1040	GGGGCCU A CAGCUUU
735	CCCGACU A UCUCGAC	1046	UACAGCU U UGADCC
737	CGACUAT C UCGACUU	1047	ACAGCUU U GAUCCU
739	ACUUCU C GACUUUG	1051	CUUUGAU C CCUGACA
744	CUCGACU U UGCGAG	1060	CCGACAU C UGGAUFC
745	UUGACUU U GCGAGU	1067	CUGGAU C UGGAGAC
753	GCGAGU C UGGGCAG	1085	GGAGCCU U UGGUUCU
763	GGCAGGU C UACUUUG	1086	GAGCCUU U GGUUCUG
765	CAGGUCU A CUUUGGG	1090	CUUUGGU U CUGGCCA
768	GUCUACU U UGGGAUC	1091	UUUGGUU C UGGOCAG
769	UCUACUU U GGGAUCA	1113	CAGGACU U GAGAAGA
775	UUGGGAU C AUUGCCC	1124	AAGACCU C ACCUAGA
778	GGAUCAU U GCGUGU	1129	CUACCU A GAAAUUG
801	CGAACAU C CAACCUU	1135	UAGAAU U GACACAA
808	CCAAACU U CCAAAC	1151	UGGACCU U AGGCCUU
809	CAACCUU C CCAACG	1152	GAACCU A GGCCUUC
820	AAAGCCU C CCGGCC	1158	UAGGCCU U CCUCUCU
833	CCCCAAU C CCUUUAU	1159	AGGCCUU C CUUCUCU
837	AADCCCU U UAUAACC	1162	CCUUCU C UCUCAG
838	AUCCCUU U AUUAACC	1164	UCCUCU C UCCAGU
839	UCCCUUU A UUAACC	1166	CCUUCU C CAGAUU
841	CCUUUAU U ACCCCCU	1174	CAGAUU U UCCAGAC
842	CUUUAU A CCCCCU	1175	AGAUUU U CCAGACU
849	AACCCCU C CUUCAGA	1176	GAUGUU C CAGACU
852	CCUCCU U CAGACAC	1183	CCAGACU U CCUGAG
853	CCUCCU C AGACACC	1184	CAGACU C CUUGAGA
863	ACACCU C AACCUU	1187	ACUUCU U CAGACAC
869	UCAACCU C UUCUGGC	1208	CAGCCU C CCAUGG
871	AACCUU U CUGGUC	1224	GCCAGCU C CCUCU
872	ACCUCU C UGGUCA	1228	GCUCU C UAUAU
878	UCUGGU C AAAAGA	1230	UCCCUU A UUAUGU
890	AGAGAAU U GGGGGU	1232	CCUCU U UAUGUU
898	GGGGGU U AGGGUG	1233	CCUCU U AUGUUG
899	GGGGCU A GGGUGG	1234	UCUAUU A UGUUGC
904	UUAGGGU C GGAACCC	1238	UUUAUGU U UGCACU
917	CCAAGCU U AGAACU	1239	UUUAUGU U GCACUG
918	CAAGCU A GAACUU	1245	UUGCACU U GUGAU
924	UAGACU U UAAGCAA	1251	UUGGAAU U AUUAU
925	AGAACU U AAGCAAC	1252	UGGAAU A UUAUA
926	GAACUU A AGCAACA	1254	UGAUU U UAUAU
945	CACACU U CGAACCC	1255	GAUAUU U AUUAU
946	ACCACU C GAAACCU	1256	AUAUU A UAUAU
959	CUGGAU U CAGGAU	1258	UAUAU U AUUAU

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1259	AUUUAUU A UUUUUUU	1440	UGUUUUU U AAAAUUU
1261	UUUUUUU U UUUUUUU	1441	GUUUUUU A AAUUUUU
1262	UUUUUUU U AUUUUUU	1446	UUAAAAU A UUAUUGU
1263	AUUUUUU A UUUUUUA	1448	AAAAUUU U AUUUUUU
1265	UUUUUUU U UUUUUUU	1449	AAAUUUU A UUUUUUU
1266	AUUUUUU U AUUUUUU	1451	AUUUUUU C UUUUUUA
1267	UUUUUUU A UUUUUUA	1456	AUUUUUU U AAGUUUU
1269	UUUUUUU U AUUUUUU	1457	UUUUUUU A AGUUUUU
1270	AUUUUUU A UUUUUUU	1461	AUUUUUU U GUUUUUU
1272	UUUUUUU U UUUUUUU	1464	AAGUUUU C UUAUUAU
1273	UUUUUUU U AUUUUUU	1466	GUUUUUU A AAUUUUU
1274	AUUUUUU A UUUUUUU	1479	UUUUUUU U UUUUUUU
1276	UUUUUUU U UUUUUUU	1480	GUUUUUU U GUUUUUU
1277	AUUUUUU U AUUUUUU	1494	CAUUUUU C AUUUUUU
1278	UUUUUUU A UUUUUUU	1498	UUUUUUU C AUUUUUU
1280	UUUUUUU U UUUUUUU	1501	CAUUUUU U GUUUUUU
1281	AUUUUUU U AUUUUUU	1512	GUUUUUU C UUUUUUU
1282	UUUUUUU A UUUUUUU	1517	UUUUUUU C UUUUUUU
1294	UUUUUUU A UUUUUUU	1528	AGUUUUU U GUUUUUU
1296	AUUUUUU U UUUUUUU	1533	GUUUUUU C UUUUUUU
1297	AUUUUUU U AUUUUUU	1537	UUUUUUU A AUUUUUU
1298	UUUUUUU A UUUUUUU	1540	UUUUUUU C GUUUUUU
1300	UUUUUUU U UUUUUUU	1546	UUUUUUU A UUUUUUU
1301	AUUUUUU U UUUUUUU	1549	GUUUUUU A UUUUUUU
1315	UUUUUUU A UUUUUUU	1551	UUUUUUU U UUUUUUU
1317	UUUUUUU C UUUUUUU	1552	UUUUUUU C UUUUUUU
1334	UUUUUUU A UUUUUUU	1566	UUUUUUU A UUUUUUU
1345	UUUUUUU U UUUUUUU	1572	UUUUUUU U UUUUUUU
1350	UUUUUUU C UUUUUUU	1576	UUUUUUU U UUUUUUU
1359	UUUUUUU U UUUUUUU	1577	UUUUUUU A UUUUUUU
1360	UUUUUUU U UUUUUUU		
1361	UUUUUUU U UUUUUUU		
1362	UUUUUUU C UUUUUUU		
1386	UUUUUUU A UUUUUUU		
1393	UUUUUUU U UUUUUUU		
1394	UUUUUUU C UUUUUUU		
1401	UUUUUUU A UUUUUUU		
1414	UUUUUUU C UUUUUUU		
1422	UUUUUUU U UUUUUUU		
1423	UUUUUUU C UUUUUUU		
1425	UUUUUUU U UUUUUUU		
1426	UUUUUUU U UUUUUUU		
1427	UUUUUUU U UUUUUUU		
1431	UUUUUUU U UUUUUUU		
1432	UUUUUUU A UUUUUUU		
1436	UUUUUUU U UUUUUUU		
1437	UUUUUUU U UUUUUUU		
1438	UUUUUUU U UUUUUUU		

Table 24: Human TNF- α Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
28	GGAAGAG CUGAUGAGGCOGAAAGGCOOGAA ACCUGCC
29	AGGAAGA CUGAUGAGGCOGAAAGGCOOGAA AACUGC
31	AGAGGA CUGAUGAGGCOGAAAGGCOOGAA AGAACU
33	UGAGAGG CUGAUGAGGCOGAAAGGCOOGAA AGAGAAC
34	GUGAGAG CUGAUGAGGCOGAAAGGCOOGAA AAGAGAA
37	UAUGUA CUGAUGAGGCOGAAAGGCOOGAA AGGAAGA
39	AGUADGU CUGAUGAGGCOGAAAGGCOOGAA AGAGGAA
44	GGGUCAG CUGAUGAGGCOGAAAGGCOOGAA AUGUGAG
58	GAGGGUG CUGAUGAGGCOGAAAGGCOOGAA AGCGUG
65	GGGGAGA CUGAUGAGGCOGAAAGGCOOGAA AGGGUGG
67	CAGGGGA CUGAUGAGGCOGAAAGGCOOGAA AGAGGGU
69	UCCAGGG CUGAUGAGGCOGAAAGGCOOGAA AGAGAGG
106	CGUCCCG CUGAUGAGGCOGAAAGGCOOGAA AUCADGC
136	UCUUGGG CUGAUGAGGCOGAAAGGCOOGAA AGCGCCU
165	CCGCCUG CUGAUGAGGCOGAAAGGCOOGAA AGCCUG
177	GAGGAAC CUGAUGAGGCOGAAAGGCOOGAA AGCACCG
180	GCUGAGG CUGAUGAGGCOGAAAGGCOOGAA ACAAGCA
181	GGCUGAG CUGAUGAGGCOGAAAGGCOOGAA AACAAGC
184	AGAGGCU CUGAUGAGGCOGAAAGGCOOGAA AGGAACA
190	AGGAGAA CUGAUGAGGCOGAAAGGCOOGAA AGGCUA
192	GAAGGAG CUGAUGAGGCOGAAAGGCOOGAA AGAGGCU
193	GGAAGGA CUGAUGAGGCOGAAAGGCOOGAA AAGAGGC
195	CAGGAAG CUGAUGAGGCOGAAAGGCOOGAA AGAAGAG
198	GAUCAGG CUGAUGAGGCOGAAAGGCOOGAA AGGAGAA
199	CGAUCAG CUGAUGAGGCOGAAAGGCOOGAA AAGGAGA
205	CUGCCAC CUGAUGAGGCOGAAAGGCOOGAA AUCAGGA
226	GGCAGAA CUGAUGAGGCOGAAAGGCOOGAA AGCGUGG
228	CAGGCAG CUGAUGAGGCOGAAAGGCOOGAA AGAGCGU
229	GCAGGCA CUGAUGAGGCOGAAAGGCOOGAA AAGAGCG
243	CACUCCA CUGAUGAGGCOGAAAGGCOOGAA AGUGCAG
244	UCACUCC CUGAUGAGGCOGAAAGGCOOGAA AAGUGCA
253	GGGGGOC CUGAUGAGGCOGAAAGGCOOGAA AUCACUC
273	CCUGGGG CUGAUGAGGCOGAAAGGCOOGAA ACUCUUC
286	UUAGAGA CUGAUGAGGCOGAAAGGCOOGAA AGGUCCC
288	GAUUGA CUGAUGAGGCOGAAAGGCOOGAA AGAGGUC
290	CUGAUUA CUGAUGAGGCOGAAAGGCOOGAA AGAGAGG
292	GGCUGAU CUGAUGAGGCOGAAAGGCOOGAA AGAGAGA
295	GAGGGCU CUGAUGAGGCOGAAAGGCOOGAA AUUAGAG
302	UGGGCCA CUGAUGAGGCOGAAAGGCOOGAA AGGGCUG

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321	AGAAGAU	CUGAUGAGGCGGAAAGGCGGAA	AUCUGAC
324	UOGAGAA	CUGAUGAGGCGGAAAGGCGGAA	ADGADCU
326	GUUOGAG	CUGAUGAGGCGGAAAGGCGGAA	AGAUGAU
327	GGUUGGA	CUGAUGAGGCGGAAAGGCGGAA	AAGAUCA
329	GGGUUUC	CUGAUGAGGCGGAAAGGCGGAA	AGAAGAU
352	CAUGGGC	CUGAUGAGGCGGAAAGGCGGAA	ACAGGCU
361	UUGCUAC	CUGAUGAGGCGGAAAGGCGGAA	ACAUGGG
364	GGUUGC	CUGAUGAGGCGGAAAGGCGGAA	ACAACAU
374	UCAGCUU	CUGAUGAGGCGGAAAGGCGGAA	AGGGUUU
391	GCCACUG	CUGAUGAGGCGGAAAGGCGGAA	AGCUGOC
421	UGGOCAG	CUGAUGAGGCGGAAAGGCGGAA	AGGGCAU
449	AGCUGGU	CUGAUGAGGCGGAAAGGCGGAA	AUCUCUC
468	GOCCUUC	CUGAUGAGGCGGAAAGGCGGAA	AUGGCAC
480	GAUGAGG	CUGAUGAGGCGGAAAGGCGGAA	ACAGGCC
484	AGUAGAU	CUGAUGAGGCGGAAAGGCGGAA	AGGUACA
487	GGEAGUA	CUGAUGAGGCGGAAAGGCGGAA	AUGAGGU
489	CUGGGAG	CUGAUGAGGCGGAAAGGCGGAA	AGAUGAG
492	GAOCUGG	CUGAUGAGGCGGAAAGGCGGAA	AGUAGAU
499	UGAAGAG	CUGAUGAGGCGGAAAGGCGGAA	ACCUGGG
502	CCUGGAA	CUGAUGAGGCGGAAAGGCGGAA	AGGAOCU
504	GOCCUUG	CUGAUGAGGCGGAAAGGCGGAA	AGAGGAC
505	GGCCUUC	CUGAUGAGGCGGAAAGGCGGAA	AAGAGGA
525	AUGGGUG	CUGAUGAGGCGGAAAGGCGGAA	AGGGGCA
538	GGGUGAG	CUGAUGAGGCGGAAAGGCGGAA	AGCICAU
541	UGGGGUU	CUGAUGAGGCGGAAAGGCGGAA	AGGGGCA
553	UGCGGCU	CUGAUGAGGCGGAAAGGCGGAA	AUGGUGU
562	AGACGGC	CUGAUGAGGCGGAAAGGCGGAA	AUGGGGC
568	GGUAGGA	CUGAUGAGGCGGAAAGGCGGAA	ACGGGCA
570	CUGGUAG	CUGAUGAGGCGGAAAGGCGGAA	AGAOGGC
573	GGUCUGG	CUGAUGAGGCGGAAAGGCGGAA	AGGAGAC
586	GGAGGUU	CUGAUGAGGCGGAAAGGCGGAA	ACCUGGG
592	CAGAGAG	CUGAUGAGGCGGAAAGGCGGAA	AGGUUGA
595	UGGCAGA	CUGAUGAGGCGGAAAGGCGGAA	AGGAGGU
597	GADGGCA	CUGAUGAGGCGGAAAGGCGGAA	AGAGGAG
604	GGCUCUU	CUGAUGAGGCGGAAAGGCGGAA	AUGGCAG
657	GGGCUCA	CUGAUGAGGCGGAAAGGCGGAA	ACCAGGG
667	CCAGAUU	CUGAUGAGGCGGAAAGGCGGAA	AUGGGCU
669	UOCCAGA	CUGAUGAGGCGGAAAGGCGGAA	AGAUGGG
671	CCUCCCA	CUGAUGAGGCGGAAAGGCGGAA	AUAGADG
682	GCUGGAA	CUGAUGAGGCGGAAAGGCGGAA	ACCCUUC
684	CAGCUGG	CUGAUGAGGCGGAAAGGCGGAA	AGACCCC
685	CCAGCUG	CUGAUGAGGCGGAAAGGCGGAA	AAGACCC
709	CAGCGCU	CUGAUGAGGCGGAAAGGCGGAA	AGUCGGU
721	GOOGAUU	CUGAUGAGGCGGAAAGGCGGAA	AUCUCAG
725	UOGGGOC	CUGAUGAGGCGGAAAGGCGGAA	AUUGAUC
735	GUOGAGA	CUGAUGAGGCGGAAAGGCGGAA	AGUCGGG
737	AAGUUGA	CUGAUGAGGCGGAAAGGCGGAA	AUAGUUG
739	CAAAGUC	CUGAUGAGGCGGAAAGGCGGAA	AGAUAGU
744	CUOGGCA	CUGAUGAGGCGGAAAGGCGGAA	AGUOGAG

745	ACUCGGC	CUGAUGAGGCGGAAAGGCGGAA	AAGUCGA
753	CUGGCCA	CUGAUGAGGCGGAAAGGCGGAA	ACUCGGC
763	CAAAGUA	CUGAUGAGGCGGAAAGGCGGAA	ACUCGGC
765	CCCAAAG	CUGAUGAGGCGGAAAGGCGGAA	AGACCUU
768	GAUCCCA	CUGAUGAGGCGGAAAGGCGGAA	AGUAGAC
769	UGAUCCC	CUGAUGAGGCGGAAAGGCGGAA	AAGUAGA
775	GGGCAAU	CUGAUGAGGCGGAAAGGCGGAA	ADCCCAA
778	ACAGGGC	CUGAUGAGGCGGAAAGGCGGAA	ADGAUCC
801	AAGGUUG	CUGAUGAGGCGGAAAGGCGGAA	ADGUUUG
808	GUUUGGG	CUGAUGAGGCGGAAAGGCGGAA	AGGUUGG
809	CGUUGGG	CUGAUGAGGCGGAAAGGCGGAA	AAGGUUG
820	GGCAGGG	CUGAUGAGGCGGAAAGGCGGAA	AGGCGUU
833	AUAAGG	CUGAUGAGGCGGAAAGGCGGAA	ADUGGGG
837	GGUAADA	CUGAUGAGGCGGAAAGGCGGAA	AGGGAUU
838	GGGUAAU	CUGAUGAGGCGGAAAGGCGGAA	AAGGGAU
839	GGGGUAA	CUGAUGAGGCGGAAAGGCGGAA	AAAGGGA
841	AGGGGGU	CUGAUGAGGCGGAAAGGCGGAA	AUAAGG
842	GAGGGGG	CUGAUGAGGCGGAAAGGCGGAA	AADAAG
849	UCUGAAG	CUGAUGAGGCGGAAAGGCGGAA	AGGGGGU
852	GUGUCUG	CUGAUGAGGCGGAAAGGCGGAA	AGGAGGG
853	GGUGUCU	CUGAUGAGGCGGAAAGGCGGAA	AAGGAGG
863	AGAGGUU	CUGAUGAGGCGGAAAGGCGGAA	AGGGUGU
869	GCCAGAA	CUGAUGAGGCGGAAAGGCGGAA	AGGUUGA
871	GAGCCAG	CUGAUGAGGCGGAAAGGCGGAA	AGAGGUU
872	UGAGCCA	CUGAUGAGGCGGAAAGGCGGAA	AAGAGGU
878	UCUUUUU	CUGAUGAGGCGGAAAGGCGGAA	AGCCAGA
890	AGCCCCC	CUGAUGAGGCGGAAAGGCGGAA	AUUUCUU
898	CGAOCUU	CUGAUGAGGCGGAAAGGCGGAA	AGCCCCC
899	COGACCC	CUGAUGAGGCGGAAAGGCGGAA	AAGCCCC
904	GGGUUCC	CUGAUGAGGCGGAAAGGCGGAA	ACCCUAA
917	AAGUUCU	CUGAUGAGGCGGAAAGGCGGAA	AGCUUGG
918	AAAGUUC	CUGAUGAGGCGGAAAGGCGGAA	AAGCUUG
924	UUGCUUA	CUGAUGAGGCGGAAAGGCGGAA	AGUUCUA
925	GUUGCUU	CUGAUGAGGCGGAAAGGCGGAA	AAGUUCU
926	UGUUGCU	CUGAUGAGGCGGAAAGGCGGAA	AAAGUUC
945	GGUUUCG	CUGAUGAGGCGGAAAGGCGGAA	AGUGGUG
946	AGGUUUC	CUGAUGAGGCGGAAAGGCGGAA	AAGUGGU
959	ADUCCUG	CUGAUGAGGCGGAAAGGCGGAA	ADCCCAG
960	CAUUCUU	CUGAUGAGGCGGAAAGGCGGAA	AADUCCA
1001	GAUUCUU	CUGAUGAGGCGGAAAGGCGGAA	AGUGGUU
1007	CAGUUUG	CUGAUGAGGCGGAAAGGCGGAA	AUUUUUA
1008	CCAGUUU	CUGAUGAGGCGGAAAGGCGGAA	AADUUCU
1021	AGUUCUG	CUGAUGAGGCGGAAAGGCGGAA	AGGCCCC
1029	COCCAGU	CUGAUGAGGCGGAAAGGCGGAA	AGUUCUG
1040	AAAGCUG	CUGAUGAGGCGGAAAGGCGGAA	AGGCCCC
1046	GGGAUCA	CUGAUGAGGCGGAAAGGCGGAA	AGCUGUA
1047	AGGGAUC	CUGAUGAGGCGGAAAGGCGGAA	AAGCUGU
1051	UGUCAGG	CUGAUGAGGCGGAAAGGCGGAA	AUCAAG
1060	GADUCCA	CUGAUGAGGCGGAAAGGCGGAA	ADGUCAG

1067	GUCUCCA	COGAUGAGGCCGAAAGGCCGAA	AUUCCAG
1085	AGAACCA	COGAUGAGGCCGAAAGGCCGAA	AGGCUCC
1086	CAGAACC	COGAUGAGGCCGAAAGGCCGAA	AAGGCUC
1090	UGGOCAG	COGAUGAGGCCGAAAGGCCGAA	ACCAAAG
1091	CUGGOC	COGAUGAGGCCGAAAGGCCGAA	AACCAAA
1113	UCUUCUC	COGAUGAGGCCGAAAGGCCGAA	AGUCCUG
1124	UCUAGGU	COGAUGAGGCCGAAAGGCCGAA	AGGUCUU
1129	CAUUC	COGAUGAGGCCGAAAGGCCGAA	AGGUGAG
1135	UUGUGUC	COGAUGAGGCCGAAAGGCCGAA	AUUUCUA
1151	AAGGCCU	COGAUGAGGCCGAAAGGCCGAA	AGGUCCA
1152	GAAGGCC	COGAUGAGGCCGAAAGGCCGAA	AAGGUCC
1158	AGAGAGG	COGAUGAGGCCGAAAGGCCGAA	AGGCCUA
1159	GAGAGAG	COGAUGAGGCCGAAAGGCCGAA	AAGGCCU
1162	CUGGAGA	COGAUGAGGCCGAAAGGCCGAA	AGGAAGG
1164	AUCUGGA	COGAUGAGGCCGAAAGGCCGAA	AGAGGAA
1166	ACAUUCG	COGAUGAGGCCGAAAGGCCGAA	AGAGAGG
1174	GUCUGGA	COGAUGAGGCCGAAAGGCCGAA	ACAUUCG
1175	AGUCUGG	COGAUGAGGCCGAAAGGCCGAA	AACAUUC
1176	AAGUCUG	COGAUGAGGCCGAAAGGCCGAA	AAACAUUC
1183	CUCAAGG	COGAUGAGGCCGAAAGGCCGAA	AGUCUGG
1184	UCUCAAG	COGAUGAGGCCGAAAGGCCGAA	AAGUCUG
1187	GUGUCUC	COGAUGAGGCCGAAAGGCCGAA	AGGAAGU
1208	CCAUUGG	COGAUGAGGCCGAAAGGCCGAA	AGGCCUG
1224	AUAGAGG	COGAUGAGGCCGAAAGGCCGAA	AGCUGGC
1228	AUAAUA	COGAUGAGGCCGAAAGGCCGAA	AGGGAGC
1230	ACAUAA	COGAUGAGGCCGAAAGGCCGAA	AGAGGGA
1232	AAACAU	COGAUGAGGCCGAAAGGCCGAA	AUAGAGG
1233	CAAACAU	COGAUGAGGCCGAAAGGCCGAA	AUAGAGG
1234	GCAAACA	COGAUGAGGCCGAAAGGCCGAA	AAAUAGA
1238	AAGUGCA	COGAUGAGGCCGAAAGGCCGAA	ACAUAAA
1239	CAAGUGC	COGAUGAGGCCGAAAGGCCGAA	AACAUAA
1245	UAUUCAC	COGAUGAGGCCGAAAGGCCGAA	AGUGCAA
1251	AAUAAAU	COGAUGAGGCCGAAAGGCCGAA	AUCACAA
1252	UAUAAAU	COGAUGAGGCCGAAAGGCCGAA	AUUCACA
1254	AAUAAUA	COGAUGAGGCCGAAAGGCCGAA	AUAUACA
1255	AAAUAAU	COGAUGAGGCCGAAAGGCCGAA	AAUAAUC
1256	UAAAUAA	COGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1258	AAUAAAU	COGAUGAGGCCGAAAGGCCGAA	AUAUAAU
1259	AAAUAAA	COGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1261	AUAUAAU	COGAUGAGGCCGAAAGGCCGAA	AUAUAAU
1262	AAUAAAU	COGAUGAGGCCGAAAGGCCGAA	AAUAAUA
1263	UAUAAAU	COGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1265	AAUAAUA	COGAUGAGGCCGAAAGGCCGAA	AUAUAAU
1266	AAAUAAU	COGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1267	UAAAUAA	COGAUGAGGCCGAAAGGCCGAA	AAAUAAA
1269	AAUAAAU	COGAUGAGGCCGAAAGGCCGAA	AUAUAAU
1270	AAAUAAA	COGAUGAGGCCGAAAGGCCGAA	AAUAAAU
1272	AUAUAAU	COGAUGAGGCCGAAAGGCCGAA	AUAUAAU
1273	AAUAAAU	COGAUGAGGCCGAAAGGCCGAA	AAUAAUA

1274	AAADAAA	CUGAUGAGGCOGAAAGGCOGAA	AAADAAU
1276	GUAAAUA	CUGAUGAGGCOGAAAGGCOGAA	AUAUAUA
1277	UGUAAAU	CUGAUGAGGCOGAAAGGCOGAA	AAUAAAU
1278	CUGUAAA	CUGAUGAGGCOGAAAGGCOGAA	AAAUAAA
1280	AUCUGUA	CUGAUGAGGCOGAAAGGCOGAA	AUAUAUA
1281	CAUCUGU	CUGAUGAGGCOGAAAGGCOGAA	AAUAAAU
1282	UCADUCU	CUGAUGAGGCOGAAAGGCOGAA	AAAUAAA
1294	AAADAAA	CUGAUGAGGCOGAAAGGCOGAA	ACADUCA
1296	CCAAAUA	CUGAUGAGGCOGAAAGGCOGAA	AUACAUA
1297	CCCAAU	CUGAUGAGGCOGAAAGGCOGAA	AAUACA
1298	UCCCAA	CUGAUGAGGCOGAAAGGCOGAA	AAAUACA
1300	UCCCCA	CUGAUGAGGCOGAAAGGCOGAA	AUAUAUA
1301	GUCUCC	CUGAUGAGGCOGAAAGGCOGAA	AAUAAAU
1315	CCCAGA	CUGAUGAGGCOGAAAGGCOGAA	ACCCCAG
1317	CCCCAG	CUGAUGAGGCOGAAAGGCOGAA	AUACCCC
1334	CAGCUC	CUGAUGAGGCOGAAAGGCOGAA	ACAUUGG
1345	CUGAGCC	CUGAUGAGGCOGAAAGGCOGAA	AGGCAGC
1350	CAUGUCU	CUGAUGAGGCOGAAAGGCOGAA	AGCCAG
1359	CAGGAA	CUGAUGAGGCOGAAAGGCOGAA	ACAUUC
1360	UCACGA	CUGAUGAGGCOGAAAGGCOGAA	AACAUU
1361	UUCACG	CUGAUGAGGCOGAAAGGCOGAA	AAACAU
1362	UUUCAG	CUGAUGAGGCOGAAAGGCOGAA	AAAACU
1386	AACAGC	CUGAUGAGGCOGAAAGGCOGAA	AUUGUC
1393	ACAUUGG	CUGAUGAGGCOGAAAGGCOGAA	ACAGUCU
1394	UACAUUG	CUGAUGAGGCOGAAAGGCOGAA	AACAGCC
1401	AGGGGC	CUGAUGAGGCOGAAAGGCOGAA	ACAUUGG
1414	AGGCAC	CUGAUGAGGCOGAAAGGCOGAA	AGGCAG
1422	UCAAAG	CUGAUGAGGCOGAAAGGCOGAA	AGGCAC
1423	AUCAA	CUGAUGAGGCOGAAAGGCOGAA	AAGGCAC
1425	UAUCA	CUGAUGAGGCOGAAAGGCOGAA	AGAAGGC
1426	AUAUCA	CUGAUGAGGCOGAAAGGCOGAA	AAGAAGG
1427	CAUAUC	CUGAUGAGGCOGAAAGGCOGAA	AAAGAAG
1431	AAAACAU	CUGAUGAGGCOGAAAGGCOGAA	AUCAA
1432	AAAACA	CUGAUGAGGCOGAAAGGCOGAA	AUCAA
1436	UUUAAA	CUGAUGAGGCOGAAAGGCOGAA	ACAUAAU
1437	UUUUA	CUGAUGAGGCOGAAAGGCOGAA	AACAUAA
1438	AUUUUA	CUGAUGAGGCOGAAAGGCOGAA	AAACAU
1439	UAUUUA	CUGAUGAGGCOGAAAGGCOGAA	AAAACAU
1440	AUAUUU	CUGAUGAGGCOGAAAGGCOGAA	AAAAACA
1441	AAUAUU	CUGAUGAGGCOGAAAGGCOGAA	AAAAAC
1446	CAGADAA	CUGAUGAGGCOGAAAGGCOGAA	AUUUUA
1448	AUCAGAU	CUGAUGAGGCOGAAAGGCOGAA	AUAUUU
1449	AAUCAGA	CUGAUGAGGCOGAAAGGCOGAA	AAUAUU
1451	UUAUCA	CUGAUGAGGCOGAAAGGCOGAA	AUAUAU
1456	ACAACUU	CUGAUGAGGCOGAAAGGCOGAA	AUCAGAU
1457	GACAACU	CUGAUGAGGCOGAAAGGCOGAA	AAUCAGA
1461	UUUAGAC	CUGAUGAGGCOGAAAGGCOGAA	ACUUAU
1464	UUGUUUA	CUGAUGAGGCOGAAAGGCOGAA	ACAACUU
1466	CAUGUU	CUGAUGAGGCOGAAAGGCOGAA	AGACAAC

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1479	GUCACCA	CUGAUGAGGCOGAAAGGCOGAA	AUCAGCA
1480	GGUCACC	CUGAUGAGGCOGAAAGGCOGAA	AADCAGC
1494	AADGAGU	CUGAUGAGGCOGAAAGGCOGAA	ACAGUUG
1498	CAGCAAU	CUGAUGAGGCOGAAAGGCOGAA	AGUGACA
1501	CCUCAGC	CUGAUGAGGCOGAAAGGCOGAA	AUGAGUG
1512	GGGAGCA	CUGAUGAGGCOGAAAGGCOGAA	AGGCUUC
1517	CCUUGGG	CUGAUGAGGCOGAAAGGCOGAA	AGCAGAG
1528	CAGACAC	CUGAUGAGGCOGAAAGGCOGAA	ACUCCUC
1533	GAUUAUA	CUGAUGAGGCOGAAAGGCOGAA	ACACAAC
1537	GGCOGAU	CUGAUGAGGCOGAAAGGCOGAA	ACAGACA
1540	GUAGGCC	CUGAUGAGGCOGAAAGGCOGAA	AUUAACG
1546	UGAUAAG	CUGAUGAGGCOGAAAGGCOGAA	AGGCOGA
1549	CACUGAA	CUGAUGAGGCOGAAAGGCOGAA	AGUAGGC
1551	GOCACUG	CUGAUGAGGCOGAAAGGCOGAA	AUAGUAG
1552	CGOCACU	CUGAUGAGGCOGAAAGGCOGAA	AADAGUA
1566	CAACCUU	CUGAUGAGGCOGAAAGGCOGAA	AUUUCUC
1572	CCUAAGC	CUGAUGAGGCOGAAAGGCOGAA	ACCUUUA
1576	CUUUCUU	CUGAUGAGGCOGAAAGGCOGAA	AGCAACC
1577	UCUUUCC	CUGAUGAGGCOGAAAGGCOGAA	AAGCAAC

Table 25: Mouse TNF-a HH Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
66	UgGAAAU a GcucCca	324	GgGUGAU C GGUCCCC
101	GGCAGGU U CUGUcCC	347	GAGAgU u cCCAaaU
101	GGCAGgU u CuGUccC	364	CCUCcCU C UcAUCAG
102	GCAGGU C UgUcCCU	366	UCcCCCU c AUCAGuu
102	gCAGgUU c ugUCCCU	366	UcCCCU C auCAGuU
106	GUUCUgU c CCUuUCA	369	CCUcAU C AGuuCUa
110	UgUcCCU u UCAcucA	376	CAGuuCU a UGGOCCA
111	gUCCCU u CaCCAC	390	AgACCCU C AcaCUcA
111	guCCCU u CACuCAc	396	ucaCAcU C AGAUCAU
112	UcCCUu C ACuCAU	401	cUCAGAU C AUCUUCU
116	UuUCACU C AcUGgcc	404	AGAUCAU C UUCUCaA
137	GCCaCAU C uCCcUCc	406	AUCAUCU U CUCaAAA
139	caCAuCU C CCUCcAg	406	AUcAUcU U cUcaAAA
177	GCAUGAU C CGcGAGG	407	UCAUCU C UCaaaau
207	AGGCaCU C CCCcAAA	409	AUCUUCU C aAAauuC
228	GGGGCUU C CAGAACU	409	AuCUuCU c AaaaUUC
228	GGGGCUU c CAGaacU	409	aUcUUCU c AAAauUc
236	CAGaacU C CAGGCGG	432	AGCCUGU A GCOCAcG
236	CAGaACU c cAGgcGg		
249	GGUGCU a UgUCUcA		
249	GGUGCU a UGucUCA	444	AcGUcGU A GCAAACC
261	UCAGCCU C UUCUCaU	501	AcGCCU C CUGGCA
261	UCAgCCU C UUCUcau	560	gGgUUGU a CCUuguC
263	AGCCUCU U CUCaUUC	560	GGguUGU A CCUugUC
263	AgCCUCU U CUcauUC	564	UGUACCU u gUCUACU
264	GCCUCU C UCauUCC	567	ACCUugU C UACUCCC
264	gCCUCU C UcauUCC	569	CUugUCU A CUCCAG
266	CCCUUCU C aUUCUUG	572	gUCUACU C CCAGGUu
269	UUCUCaU U CCUGcUu	572	GUcUacU c CCAGguu
270	UUCCaU C CUGcUuG	572	GuCUacU C CCAGGUu
276	UCCUGcU u GUGGAG	579	CCCAGGU u CUUUCA
297	CCACGCU C UUCUGuC	580	CCAGguU c uCUUcAa
299	ACGCUCU U CUGuCUa	580	CCaGGuU c UCuUcaa
300	CGCCCU C UGUcUaC	582	AGGUUCU C UUCaagg
304	CUuCUgU c uAcUGaa	582	AGGUuCU C UUCAAGG
306	UcUGUcU a cUgAAcU	584	GUuCUU U CAAGGGa
314	CUGaACU U cGGgGUG	585	UuCUU C AAGGGaC
315	UGaACU c GGgGUGA	608	CcCGaCU a CgugCUC
315	uGaACU c GGGguGa	615	aCgUGcU C CUCAcCC
324	gGGUGaU c GgUCCcC	615	AcGUGCU C CUCAcCC
		618	UGCUUCU C AOCACA

630	ACACCGU C AGCCGau	940	GUCUACU c cUCAGaG
630	ACACCGU C AgCCGaU	943	UACUccU C AGaGcCc
638	agcCgAU u uGCUaUc	972	UCUazCU u AgAAAGg
643	aUUUGcU a uCUcAuA	972	ucUazCU u AGAaAgG
645	UuGCUaU C UCauUACC	973	CUaACuU A GAAAggG
647	GCuaUCU C aUAACCAG	984	AGgGgAU U auGGcuc
663	agAAaGU C AACCUCC	984	AGGGgaU U aUGgCUc
669	UCAACCU C CUCUCUG	985	GGGauU a uGGcUCa
669	UcAAccU c cUcUCUG	997	UcAGaGU c CAACucU
672	ACCUCCU C UCUGCCg	1010	CugUGCU c AGAgCUU
674	CUCUCU C UGCCgUC	1017	cAGAgCU U UcaACAA
681	cUGCCgU C AagaGcC	1018	AGAgCUU U cAAcAAC
681	CUGCCgU C AAGAGCC	1019	GAgCUUU c AaCAACu
681	CUGcCgU C aagAgcC	1073	UgGGCCU c ucAUgCA
734	CCCUGGU A UGAGCCC	1096	AAGgAcU C AaAugGG
734	CccUGGU a ugaGCCc	1106	aUGGGcU U uccGAU
744	AGCCCAU a UAcCUGG	1107	UGGGcUU u ccGAUu
746	CCCAUaU A cCUGGGA	1108	GGgCUUU c cGzaUUC
759	GAgGAGU C uuCCAGc	1115	CcGAauU C ACUGGaG
759	GAGGaGU C UUCcAGC	1133	CGAAugU C CAuuCcU
761	GGaGUUU U CCAGCUG	1164	gagUGgU c AgGUUGc
762	GaGUUUU C CAGCUGG	1180	UcUgUcU c agaAUGA
786	ACCaACU C AGCGCUG	1203	zaGAuCU c AGGCCUU
798	CUGAGgU C AAUcUGC	1210	cAGGCCU U CCUzccU
802	GgUCAAU C uGCCCaA	1211	AGGCCUU C CUacCUu
812	CCCaAgU A cuUaGAC	1214	CCUCCU a cCUuCAG
816	AgUAcuU a GACUUG	1218	CcuACcU u CaGACCU
821	uUaGACU U UGCGAG	1218	CCzaCCU U CAGACcu
822	UaGACUU U GCgGAGU	1218	cCuACcU u cAgACCU
830	GCgGAGU C cGGGCAG	1218	CCUacCU u CAGAccU
840	GGCAGGU C UACUUG	1219	CuaCCUU C AGACcuu
842	CAGGCUU A CUUUGGa	1219	CuAcCUU c agACcUU
842	CAGgucU a CUUugGA	1226	CagACCU U uCCAGAC
842	cagGuCU a CUUUGGA	1226	CAGAccU U UCCAGAC
845	GUCUACU U UGGagUC	1227	agACCUU u CCAGACu
846	UCUACUU U GGagUCA	1227	AGAccUU U CCAGACU
852	UUGGagU C AUUGCuC	1228	GAccUUU C CAGACTc
855	GagUCAU U GCuUGU	1238	gACUcuU c cCUGAGG
887	AUCCaUU c ucUAACC	1262	CAGCCuU C CuCAcG
891	AuuCuCU a CCCaGCC	1283	CCCCccU C uaUUUAU
905	CCcCaCU C UgaCCCC	1283	cCccCCU C UAUUUAU
905	cCCCaCU c UgaCCCC	1285	cCCCUU A UUUUAuU
905	CcCCACU c uGAccCC	1287	CcuCUAU u UauAuUU
914	GAcCCcU U uacUCUG	1287	CCUCUAD U UAUaUUU
915	ACCCCUU u acUCuGA	1288	CGCUADU U AUaUUUG
919	CUUUAcU c ugaCCcC	1289	UCUADUU A UaUUUGC
928	GACCCcU u UaUugUC	1293	UUUAUaU U UGCACUU
928	gAcCCCU U UAUUgUC	1293	uUUaUaU u UGcAcUu
932	CCUUUAU U guCUaCU	1294	UUUAaUU U GCACUa

1300	UUGCACU U aUuAUu	1462	aCCuUGU u GCUCCU
1303	CACuUaU u AuUuAUU	1470	GccuCCU C UUUUGcU
1304	acOuAUU A UUUADUA	1472	cuCCUCU U UUGcUUA
1306	UuAUUAU U UAUUAUU	1473	uCCUCUU U UGcUUAU
1307	uAUUAUU U AUUAUUU	1474	CcUCUUU U GcUUADG
1307	UaUUAUU U AuUAUUU	1478	UUUGcU U ADGUUa
1308	AOUAUUU A UUAUUUA	1479	UUUGcUU a UGuuAa
1310	UauUUAU U AUUAUUU	1479	UUUGcUU A UGUUUaa
1310	UADUUAU U AUUAUUU	1484	UUADGUU U aaaAcAA
1310	UADUUAU U AUUAUUU	1498	AAAUuuU U ADUUAAC
1311	ADUUAUU A UUAUUUU	1511	AcccAaU U GCUUAA
1311	ADUUAUU A UUAUUUU	1514	cAaUUGU C UuAAuAA
1311	AuuUAUU A UuUuuUU	1516	aUUGUCU u AAuAAcG
1313	UUADUUAU U UAUUAUU	1529	CgcugAU u UGUUGAC
1313	UUADUUAU U UAUUAUU	1529	cGUUGAU U UGUUGAC
1313	uUADUUAU u UauUUAu	1530	gCUGAUU u gGUgacC
1314	UADUUAU U AUUAUUU	1530	GCUGAUU U GGUgACC
1314	UADUUAU U AUUAUUU	1563	UgaACCU c UGcUCCC
1315	ADUUAUU A UUAUUUA	1563	ugaaCCU C UGCUCCC
1317	UADUUAU U UAUUAUU	1568	CUCUGCU C CCCAcGG
1318	ADUUAUU U AUUAUUU	1589	UGaCUGU A AUuGcCC
1319	UUUAUUU A UUAUUUA	1592	CUGUAAU u GcCCUAC
1326	ADUUAUU A UUAUUUU	1617	GAGAAAU A AAGaUcG
1328	UADUUAU U UAUUUgC	1623	UAAAGaU c GCUUAaa
1329	ADUUAUU U AUUUgCu	1633	UUAAAAU a aaAAACC
1330	UUUAUUU A UUUgCu	25	AgGgaCU a gCCagGA
1332	UADUUAU U UgCuUAU		
1333	AUUUAUU U gCuUAUG		
1337	auUUGCU U AuGAuUG		
1338	uUUGCUU A uGAuUGu		
1346	UGAUUGU A UUAUUUU		
1348	AAUGUAU U UAUUUGG		
1349	ADGUUAU U AUUUGGa		
1350	UGUAUUU A UUUUGGa		
1352	uAUuUAU u UGGaAGG		
1352	UADUUAU U UGGaAGg		
1353	AUUUAUU U GGaAGgC		
1369	GGGUgU C CUGGaGG		
1398	gCUguCU U cAGACag		
1398	GCUGaCU U cagaCAG		
1412	GACAUUGU U UUCuGUG		
1413	ACAUUGU U UCuGUGA		
1414	CAUGUUU U CuGUGAA		
1415	AUGUUUU C uGUGAAA		
1415	AUGUUUU c UgugAaA		
1438	gaGCGU c CCCAccU		
1451	CUGGCUU C UcUaCCU		
1453	ggCCUCU C UaCCuUG		

Table 26: Mouse TNF- α Hammerhead Ribozyme Sequences

nt. Position	Mouse HH Ribozyme Sequence
25	UCCUGGC CUGAUGAGGCOGAAAGGCOGAA AGUCCCU
66	UGGGAGC CUGAUGAGGCOGAAAGGCOGAA AUUUCCA
101	GGGACAG CUGAUGAGGCOGAAAGGCOGAA ACCUGCC
101	GGGACAG CUGAUGAGGCOGAAAGGCOGAA ACCUGCC
102	AGGGACA CUGAUGAGGCOGAAAGGCOGAA AACUUGC
102	AGGGACA CUGAUGAGGCOGAAAGGCOGAA AACUUGC
106	UGAAAGG CUGAUGAGGCOGAAAGGCOGAA ACAGAAC
110	UGAGUGA CUGAUGAGGCOGAAAGGCOGAA AGGGACA
111	GUGAGUG CUGAUGAGGCOGAAAGGCOGAA AAGGGAC
111	GUGAGUG CUGAUGAGGCOGAAAGGCOGAA AAGGGAC
112	AGUGAGU CUGAUGAGGCOGAAAGGCOGAA AAAGGGA
116	GGCCAGU CUGAUGAGGCOGAAAGGCOGAA AGUGAAA
137	GGAGGGA CUGAUGAGGCOGAAAGGCOGAA AUGUGGC
139	CUGGAGG CUGAUGAGGCOGAAAGGCOGAA AGAUGUG
177	CGUCGCG CUGAUGAGGCOGAAAGGCOGAA AUCAUGC
207	UUUGGGG CUGAUGAGGCOGAAAGGCOGAA AGUGCCU
228	AGUUCUG CUGAUGAGGCOGAAAGGCOGAA AAGCCCC
228	AGUUCUG CUGAUGAGGCOGAAAGGCOGAA AAGCCCC
236	CCGCCUG CUGAUGAGGCOGAAAGGCOGAA AGUUCUG
236	CCGCCUG CUGAUGAGGCOGAAAGGCOGAA AGUUCUG
249	UGAGACA CUGAUGAGGCOGAAAGGCOGAA AGGCACC
249	UGAGACA CUGAUGAGGCOGAAAGGCOGAA AGGCACC
261	AUGAGAA CUGAUGAGGCOGAAAGGCOGAA AGGCUGA
261	AUGAGAA CUGAUGAGGCOGAAAGGCOGAA AGGCUGA
263	GAAUGAG CUGAUGAGGCOGAAAGGCOGAA AGAGGCU
263	GAAUGAG CUGAUGAGGCOGAAAGGCOGAA AGAGGCU
264	GGAUGA CUGAUGAGGCOGAAAGGCOGAA AAGAGGC
264	GGAUGA CUGAUGAGGCOGAAAGGCOGAA AAGAGGC
266	CAGGAU CUGAUGAGGCOGAAAGGCOGAA AGAAGAG
269	AAGCAGG CUGAUGAGGCOGAAAGGCOGAA AUGAGAA
270	CAAGCAG CUGAUGAGGCOGAAAGGCOGAA AADGAGA
276	CUGCCAC CUGAUGAGGCOGAAAGGCOGAA AGCAGGA
297	GACAGAA CUGAUGAGGCOGAAAGGCOGAA AGCGUGG
299	UAGACAG CUGAUGAGGCOGAAAGGCOGAA AGAGCGU
300	GUAGACA CUGAUGAGGCOGAAAGGCOGAA AAGAGCG
304	UUCAGUA CUGAUGAGGCOGAAAGGCOGAA ACAGAGG
306	AGUUCAG CUGAUGAGGCOGAAAGGCOGAA AGACAGA
314	CACCCCG CUGAUGAGGCOGAAAGGCOGAA AGUUCAG
315	UCACCCC CUGAUGAGGCOGAAAGGCOGAA AAGUUCA

315	UACCCC	CUGAUGAGGCGGAAAGGCGGAA	AAGJUCA
324	GGGGACC	CUGAUGAGGCGGAAAGGCGGAA	AUCACCC
324	GGGGACC	CUGAUGAGGCGGAAAGGCGGAA	AUCACCC
347	ADUUGGG	CUGAUGAGGCGGAAAGGCGGAA	ACTUCUC
364	CTGAUGA	CUGAUGAGGCGGAAAGGCGGAA	AGGGAGG
366	AACUGAU	CUGAUGAGGCGGAAAGGCGGAA	AGAGGGA
366	AACUGAU	CUGAUGAGGCGGAAAGGCGGAA	AGAGGGA
369	UAGAACU	CUGAUGAGGCGGAAAGGCGGAA	ADGAGAG
376	UGGGCCA	CUGAUGAGGCGGAAAGGCGGAA	AGAACTG
390	UGAGGCU	CUGAUGAGGCGGAAAGGCGGAA	AGGGGCU
396	ADGAGCU	CUGAUGAGGCGGAAAGGCGGAA	AGUGUGA
401	AGAAGAU	CUGAUGAGGCGGAAAGGCGGAA	AUCGAGG
404	UUGAGAA	CUGAUGAGGCGGAAAGGCGGAA	ADGAGCU
406	UUUUGAG	CUGAUGAGGCGGAAAGGCGGAA	AGAUAGU
406	UUUUGAG	CUGAUGAGGCGGAAAGGCGGAA	AGAUAGU
407	AUUUGA	CUGAUGAGGCGGAAAGGCGGAA	AAGAUGA
409	GAUUUU	CUGAUGAGGCGGAAAGGCGGAA	AGAAGAU
409	GAUUUU	CUGAUGAGGCGGAAAGGCGGAA	AGAAGAU
409	GAUUUU	CUGAUGAGGCGGAAAGGCGGAA	AGAAGAU
432	CGUGGGC	CUGAUGAGGCGGAAAGGCGGAA	ACAGGCU
444	GGUUGC	CUGAUGAGGCGGAAAGGCGGAA	AGGACGU
501	UGGOCAG	CUGAUGAGGCGGAAAGGCGGAA	AGGGGCU
560	GACAAGG	CUGAUGAGGCGGAAAGGCGGAA	ACAAACC
560	GACAAGG	CUGAUGAGGCGGAAAGGCGGAA	ACAAACC
564	AGUAGAC	CUGAUGAGGCGGAAAGGCGGAA	AGGUACA
567	GGGAGUA	CUGAUGAGGCGGAAAGGCGGAA	ACAAGGU
569	CUGGAG	CUGAUGAGGCGGAAAGGCGGAA	AGACAGG
572	AACUGG	CUGAUGAGGCGGAAAGGCGGAA	AGUAGAC
572	AACUGG	CUGAUGAGGCGGAAAGGCGGAA	AGUAGAC
572	AACUGG	CUGAUGAGGCGGAAAGGCGGAA	AGUAGAC
579	UGAAGAG	CUGAUGAGGCGGAAAGGCGGAA	ACCUGGG
580	UUGAGA	CUGAUGAGGCGGAAAGGCGGAA	AACUGG
580	UUGAGA	CUGAUGAGGCGGAAAGGCGGAA	AACUGG
582	CCUUGAA	CUGAUGAGGCGGAAAGGCGGAA	AGAACCU
582	CCUUGAA	CUGAUGAGGCGGAAAGGCGGAA	AGAACCU
584	UCCCUUG	CUGAUGAGGCGGAAAGGCGGAA	AGAGAAC
585	GUCUUU	CUGAUGAGGCGGAAAGGCGGAA	AMAGAA
608	GAGCAG	CUGAUGAGGCGGAAAGGCGGAA	AGUUGGG
615	GGGUGAG	CUGAUGAGGCGGAAAGGCGGAA	AGCAGGU
615	GGGUGAG	CUGAUGAGGCGGAAAGGCGGAA	AGCAGGU
618	UGUGGU	CUGAUGAGGCGGAAAGGCGGAA	AGGAGCA
630	AUGGCU	CUGAUGAGGCGGAAAGGCGGAA	ACGGUGU
630	AUGGCU	CUGAUGAGGCGGAAAGGCGGAA	ACGGUGU
638	GATAGCA	CUGAUGAGGCGGAAAGGCGGAA	AUGGCU
643	UADGAGA	CUGAUGAGGCGGAAAGGCGGAA	AGCAAAU
645	GGUADGA	CUGAUGAGGCGGAAAGGCGGAA	ADAGCAA
647	CUGGUAD	CUGAUGAGGCGGAAAGGCGGAA	AGAUAGC

663	GGAGGUU	CUGAUGAGGCGAAAGGCGAA	ACUUCU
669	CAGAGAG	CUGAUGAGGCGAAAGGCGAA	AGGUGA
669	CAGAGAG	CUGAUGAGGCGAAAGGCGAA	AGGUGA
672	CGGCAGA	CUGAUGAGGCGAAAGGCGAA	AGGAGU
674	GAOGGCA	CUGAUGAGGCGAAAGGCGAA	AGAGGAG
681	GGUCUU	CUGAUGAGGCGAAAGGCGAA	ACGGCAG
681	GGUCUU	CUGAUGAGGCGAAAGGCGAA	ACGGCAG
681	GGUCUU	CUGAUGAGGCGAAAGGCGAA	ACGGCAG
734	GGGCUCA	CUGAUGAGGCGAAAGGCGAA	ACCAGGG
734	GGGCUCA	CUGAUGAGGCGAAAGGCGAA	ACCAGGG
744	CCAGGUA	CUGAUGAGGCGAAAGGCGAA	AUGGGCU
746	UCCAGG	CUGAUGAGGCGAAAGGCGAA	AUAAGGG
759	GUUGGA	CUGAUGAGGCGAAAGGCGAA	ACUCCUC
759	GUUGGA	CUGAUGAGGCGAAAGGCGAA	ACUCCUC
761	CAGCUG	CUGAUGAGGCGAAAGGCGAA	AGACCC
762	CCAGCUG	CUGAUGAGGCGAAAGGCGAA	AAGACUC
786	CAGCGU	CUGAUGAGGCGAAAGGCGAA	AGUUGGU
798	GCAGAU	CUGAUGAGGCGAAAGGCGAA	ACCUAG
802	UUGGCA	CUGAUGAGGCGAAAGGCGAA	AUUGACC
812	GUUAAG	CUGAUGAGGCGAAAGGCGAA	ACUUGG
816	CAAAGUC	CUGAUGAGGCGAAAGGCGAA	AGUACU
821	CUCCGA	CUGAUGAGGCGAAAGGCGAA	AGUCUA
822	ACUCCG	CUGAUGAGGCGAAAGGCGAA	AAGUCUA
830	CUCCCG	CUGAUGAGGCGAAAGGCGAA	ACUCCG
840	CAAAGUA	CUGAUGAGGCGAAAGGCGAA	ACCUCC
842	UCCAAAG	CUGAUGAGGCGAAAGGCGAA	AGACCC
842	UCCAAAG	CUGAUGAGGCGAAAGGCGAA	AGACCC
842	UCCAAAG	CUGAUGAGGCGAAAGGCGAA	AGACCC
845	GAUCUA	CUGAUGAGGCGAAAGGCGAA	AGUAGAC
846	UGACUC	CUGAUGAGGCGAAAGGCGAA	AAGUAGA
852	GAUCAU	CUGAUGAGGCGAAAGGCGAA	ACUCCAA
855	ACAGAGC	CUGAUGAGGCGAAAGGCGAA	ADGACUC
887	GGUAGA	CUGAUGAGGCGAAAGGCGAA	AAGGUAU
891	GGUUGG	CUGAUGAGGCGAAAGGCGAA	AGAGAUA
905	GGGCUA	CUGAUGAGGCGAAAGGCGAA	AGUGGGG
905	GGGCUA	CUGAUGAGGCGAAAGGCGAA	AGUGGGG
905	GGGCUA	CUGAUGAGGCGAAAGGCGAA	AGUGGGG
914	CAGAGUA	CUGAUGAGGCGAAAGGCGAA	AGGGGUC
915	UCAGAGU	CUGAUGAGGCGAAAGGCGAA	AAGGGGU
919	GGGCUA	CUGAUGAGGCGAAAGGCGAA	AGUAAAG
928	GACAAUA	CUGAUGAGGCGAAAGGCGAA	AGGGGUC
928	GACAAUA	CUGAUGAGGCGAAAGGCGAA	AGGGGUC
932	AGUAGAC	CUGAUGAGGCGAAAGGCGAA	AUAAGG
940	CUUGAG	CUGAUGAGGCGAAAGGCGAA	AGUAGAC
943	GGGCUU	CUGAUGAGGCGAAAGGCGAA	AGGAGUA
972	CCUUCU	CUGAUGAGGCGAAAGGCGAA	AGUAGA
972	CCUUCU	CUGAUGAGGCGAAAGGCGAA	AGUAGA
973	CCUUCU	CUGAUGAGGCGAAAGGCGAA	AAGUAG
984	GAGCAU	CUGAUGAGGCGAAAGGCGAA	AUCCCU

984	GAGCCAU	CUGAUGAGGCOGAAAGGCOGAA	AUCCCCU
985	UGAGCCA	CUGAUGAGGCOGAAAGGCOGAA	AAUCCCC
997	AGAGUUG	CUGAUGAGGCOGAAAGGCOGAA	ACUCUGA
1010	AAGCUCU	CUGAUGAGGCOGAAAGGCOGAA	AGCACAG
1017	UUGUUGA	CUGAUGAGGCOGAAAGGCOGAA	AGCUCUG
1018	GUUGUUG	CUGAUGAGGCOGAAAGGCOGAA	AAGCUCU
1019	AGUUGUU	CUGAUGAGGCOGAAAGGCOGAA	AAAGCUC
1073	UGCAUGA	CUGAUGAGGCOGAAAGGCOGAA	AGGCCCA
1096	CCCAUUG	CUGAUGAGGCOGAAAGGCOGAA	AGUCCUU
1106	AUUCGGA	CUGAUGAGGCOGAAAGGCOGAA	AGCCCAU
1107	AAUUCGG	CUGAUGAGGCOGAAAGGCOGAA	AAGCCCA
1108	GAUUCGG	CUGAUGAGGCOGAAAGGCOGAA	AAAGCCC
1115	CCCAUGU	CUGAUGAGGCOGAAAGGCOGAA	AAUUCGG
1133	AGGAADG	CUGAUGAGGCOGAAAGGCOGAA	ACAUUCG
1164	GCAACCU	CUGAUGAGGCOGAAAGGCOGAA	ACCAUCU
1180	UCAUUCU	CUGAUGAGGCOGAAAGGCOGAA	AGACAGA
1203	AAGGCCU	CUGAUGAGGCOGAAAGGCOGAA	AGAUCCU
1210	AGGUAGG	CUGAUGAGGCOGAAAGGCOGAA	AGGCCUG
1211	AAGGUAG	CUGAUGAGGCOGAAAGGCOGAA	AAGGCCU
1214	CUGAAGG	CUGAUGAGGCOGAAAGGCOGAA	AGGAAGG
1218	AGGUUCG	CUGAUGAGGCOGAAAGGCOGAA	AGGUAGG
1218	AGGUUCG	CUGAUGAGGCOGAAAGGCOGAA	AGGUAGG
1218	AGGUUCG	CUGAUGAGGCOGAAAGGCOGAA	AGGUAGG
1218	AGGUUCG	CUGAUGAGGCOGAAAGGCOGAA	AGGUAGG
1219	AAGGUUC	CUGAUGAGGCOGAAAGGCOGAA	AAGGUAG
1219	AAGGUUC	CUGAUGAGGCOGAAAGGCOGAA	AAGGUAG
1226	GUCUGGA	CUGAUGAGGCOGAAAGGCOGAA	AGGUUCG
1226	GUCUGGA	CUGAUGAGGCOGAAAGGCOGAA	AGGUUCG
1227	AGUCUGG	CUGAUGAGGCOGAAAGGCOGAA	AAGGUUC
1227	AGUCUGG	CUGAUGAGGCOGAAAGGCOGAA	AAGGUUC
1228	GAGUCUG	CUGAUGAGGCOGAAAGGCOGAA	AAAGGUC
1238	CCUCAGG	CUGAUGAGGCOGAAAGGCOGAA	AAGAGUC
1262	CUGUGAG	CUGAUGAGGCOGAAAGGCOGAA	AAGGCUG
1283	AUAADA	CUGAUGAGGCOGAAAGGCOGAA	AGGGGGG
1283	AUAADA	CUGAUGAGGCOGAAAGGCOGAA	AGGGGGG
1285	AUAADA	CUGAUGAGGCOGAAAGGCOGAA	AGAGGGG
1287	AAUAADA	CUGAUGAGGCOGAAAGGCOGAA	AUAGAGG
1287	AAUAADA	CUGAUGAGGCOGAAAGGCOGAA	AUAGAGG
1288	CAUAADA	CUGAUGAGGCOGAAAGGCOGAA	AUAAGAG
1289	GCAADA	CUGAUGAGGCOGAAAGGCOGAA	AAUAGA
1293	AAGUGCA	CUGAUGAGGCOGAAAGGCOGAA	AUAADA
1293	AAGUGCA	CUGAUGAGGCOGAAAGGCOGAA	AUAADA
1294	UAAGUGC	CUGAUGAGGCOGAAAGGCOGAA	AAUAADA
1300	AAUAADA	CUGAUGAGGCOGAAAGGCOGAA	AGUGCAA
1303	AAUAADA	CUGAUGAGGCOGAAAGGCOGAA	AUAAGUG
1304	UAUAADA	CUGAUGAGGCOGAAAGGCOGAA	AAUAAGU
1306	AAUAADA	CUGAUGAGGCOGAAAGGCOGAA	AUAADA
1307	AAUAADA	CUGAUGAGGCOGAAAGGCOGAA	AAUAADA
1307	AAUAADA	CUGAUGAGGCOGAAAGGCOGAA	AAUAADA

1308	UAAAUAA	CUGAUGAGGCGGAAAGGCGGAA	AAAUAAU
1310	AAUAAAU	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1310	AAUAAAU	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1310	AAUAAAU	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1311	AAAUAAA	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1311	AAAUAAA	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1311	AAAUAAA	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1311	AAAUAAA	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1313	AUAUAAU	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1313	AUAUAAU	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1313	AUAUAAU	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1313	AUAUAAU	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1314	AAUAAAU	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1314	AAUAAAU	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1315	UAAUAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAUAAU
1317	AAUAAUA	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1318	AAUAAAU	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1319	UAAAUAA	CUGAUGAGGCGGAAAGGCGGAA	AAAUAAA
1326	AAAUAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAUAAU
1328	GCAUAAU	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1329	AGCAAAU	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1330	AAGCAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAUAAA
1332	AUAAGCA	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1333	CAUAAGC	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1337	CAUUCAU	CUGAUGAGGCGGAAAGGCGGAA	AGCAAAU
1338	ACAUAUA	CUGAUGAGGCGGAAAGGCGGAA	AAGCAAA
1346	AAAUAAA	CUGAUGAGGCGGAAAGGCGGAA	ACAUAUA
1348	CCAUAUA	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1349	UCCAUAU	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1350	UUCCAUA	CUGAUGAGGCGGAAAGGCGGAA	AAAUAAA
1352	CCUCCA	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1352	CCUCCA	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAU
1353	GCCUCC	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1369	CCUCCAG	CUGAUGAGGCGGAAAGGCGGAA	ACAUAUA
1398	CUGUCUG	CUGAUGAGGCGGAAAGGCGGAA	AGACAGC
1398	CUGUCUG	CUGAUGAGGCGGAAAGGCGGAA	AGACAGC
1412	CACAGAA	CUGAUGAGGCGGAAAGGCGGAA	ACAUAUA
1413	UACAGAA	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1414	UUACAG	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1415	UUUACA	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1415	UUUACA	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1438	AGGUGGG	CUGAUGAGGCGGAAAGGCGGAA	ACAUAUA
1451	AGGUAGA	CUGAUGAGGCGGAAAGGCGGAA	AGGUCAG
1453	CAAGGUA	CUGAUGAGGCGGAAAGGCGGAA	AGGUGGC
1455	AACAAGG	CUGAUGAGGCGGAAAGGCGGAA	AGGAGAG
1462	AGGAGGC	CUGAUGAGGCGGAAAGGCGGAA	ACAUAUA
1470	AGCAAAA	CUGAUGAGGCGGAAAGGCGGAA	AGGAGGC
1472	UAAGCAA	CUGAUGAGGCGGAAAGGCGGAA	AGGAGAG
1473	AUAAGCA	CUGAUGAGGCGGAAAGGCGGAA	AAGAGGA
1474	CAUAAGC	CUGAUGAGGCGGAAAGGCGGAA	AAUAAAU
1478	UAAACAU	CUGAUGAGGCGGAAAGGCGGAA	AGCAAAA

1479	UUAACA	CUGAUGAGGCGGAAAGGCGGAA	AAGCAAA
1479	UUAACA	CUGAUGAGGCGGAAAGGCGGAA	AAGCAAA
1484	UUGUUU	CUGAUGAGGCGGAAAGGCGGAA	AACUUA
1498	GUUAGU	CUGAUGAGGCGGAAAGGCGGAA	AATUUU
1511	UUAAGC	CUGAUGAGGCGGAAAGGCGGAA	AUUGGU
1514	UUAUUA	CUGAUGAGGCGGAAAGGCGGAA	ACAADU
1516	CGUUUU	CUGAUGAGGCGGAAAGGCGGAA	AGACAU
1529	GUACCA	CUGAUGAGGCGGAAAGGCGGAA	AUCAGG
1529	GUACCA	CUGAUGAGGCGGAAAGGCGGAA	AUCAGG
1530	GGUACC	CUGAUGAGGCGGAAAGGCGGAA	AADUAG
1530	GGUACC	CUGAUGAGGCGGAAAGGCGGAA	AADUAG
1563	GGGAGC	CUGAUGAGGCGGAAAGGCGGAA	AGGUUA
1563	GGGAGC	CUGAUGAGGCGGAAAGGCGGAA	AGGUUA
1568	CGUGGG	CUGAUGAGGCGGAAAGGCGGAA	AGCAGG
1589	GGGCAU	CUGAUGAGGCGGAAAGGCGGAA	ACAGUA
1592	GUAGGC	CUGAUGAGGCGGAAAGGCGGAA	AUUACG
1617	CGAUUU	CUGAUGAGGCGGAAAGGCGGAA	AUUUCU
1623	UUUAAG	CUGAUGAGGCGGAAAGGCGGAA	AUUUUA
1633	GGUUUU	CUGAUGAGGCGGAAAGGCGGAA	AUUUUA

Table 27: Human TNF- α Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
46	AGCCUUGG AGAA GUUUGU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	ACUAUCU GAC CCACGGCU
54	GAGGUGUG AGAA GUGUGU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	ACCCACG GCU CCACCCUC
185	GGAGAAAG AGAA GAGGAA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UUUCCUA GGC UCUUCUCC
201	CUCCACAG AGAA GGAAGG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CCUUCUU GAU GGUUGGAG
230	GUCCAGCA AGAA GAAGAG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CUUUCUU GGC UGCUGCAC
234	CAGAUGCC AGAA GGCAGA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UCUUGCU GCU GCAUUCUG
254	CTUUGGG AGAA GAUCAC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GUUAUCG GGC CCCAGAGG
296	GGCCAGAG AGAA GAUUG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CUUAUCA GGC CUCUGGCC
317	AGAAAGUG AGAA GACUGC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GCAGUCA GAU CAUCUUCU
387	GCCACUGG AGAA GGGCCU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AGGGGCA GCU CCAGUGGC
404	AUUGGCC AGAA GUUCAG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CUAACC GGC GGGCCAAU
453	GCACCACC AGAA GGUUUV ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AUAACCA GCU GGUUGGUC
518	GUUGGAGG AGAA GCGUUG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CAAGGCU GGC CUUCCACC
554	GGGUAUGC AGAA GAUGGU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	ACCAUCA GGC GCAUUGCC
565	UGGUAGGA AGAA GCGAUG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CAUGGCC GUC UCCUACCA
576	UQACCTUG AGAA GGUAGG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CCUACCA GAC CAAGGUCA
607	CCUUCUCC AGAA GGAAGA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UCUUGCA GCU GGAQNAAG
704	AGGCTUGA AGAA GUCACC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GGUGAGC GAC UCAAGCCU
726	GAUAGUCC AGAA GAUUGA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UCAUUCG GGC CGACTAUC
730	UCGAGUA AGAA GGGCGA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UUGGGCC GAC UAUCUUGA
824	GGGAUUGG AGAA GGGGAG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CUUCCCU GGC CCAUUGCC
1042	GGGAUUC AGAA GUAGGC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GCCUACA GCU UUGAUCCC
1168	CUAGAAC AGAA GGAAGG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CUUUGCA GAU GUUUCUAG
1178	UCAAGGAA AGAA GGAAGC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GUUUGCA GAC UUGCUUGA
1202	AUGGGGAG AGAA GGGGUC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GAGCCCA GGC CUUCCCAU
1220	AUAAGGG AGAA GGCUGC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GGAGCCA GCU CCCUCUUA
1284	AUACAUUC AGAA GUAAAU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AUUUACA GAU GAAUGUAU
1340	UAGGCCAA AGAA GCUCCU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AGGAGCU GGC UUGGCUCA
1390	UACAUGGG AGAA GCUUUA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AUAGGCU GUU CCCAUGUA

1452	ACAACTUA	AGAA	GAUAAU	ACCAGAGAAACACACGCTUGGUA	CAUUAUACCUUGUA	AUUAUCU	GAU	UAGUUGU
1475	GUACACCA	AGAA	GCAUUG	ACCAGAGAAACACACGCTUGGUA	CAUUAUACCUUGUA	CAAUUCU	GAU	UUGGUGAC
1513	CCUUGGGG	AGAA	GAGGCC	ACCAGAGAAACACACGCTUGGUA	CAUUAUACCUUGUA	GGCCUCU	GCU	CCCCAGGG
1541	GAUAGUA	AGAA	GAUUAU	ACCAGAGAAACACACGCTUGGUA	CAUUAUACCUUGUA	GUAAUUG	GCC	UACUAUUC

SUBSTITUTE SHEET (RULE 26)

Table 28: Mouse TNF- α Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
103	GUUAAAG AGAA GAACTU ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	AGGUUU GUC CUUUUAC
256	UUAGAAAG AGAA GAGACA ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	UGUUUA GUC CUUUUUA
272	CUUCCACA AGAA GGAUUG ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	CAUUUU GUC UGUGGAG
301	GUUUAAGU AGAA GAAAGG ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	CUUUUU GUC UACUGAC
325	CUUUUGGG AGAA GAUAC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC CUUAAAG
370	GUUUAAGU AGAA GAUAG ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	CUUUAU GUC CUUUGGC
383	GUUUAAGU AGAA GGGCCA ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	UGUUUA GUC CUUUAAC
397	AGAAAGU AGAA GAGUGU ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	ACAUUA GUC CUUUUUU
467	GUUUAAGU AGAA GGUUUU ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	AGAGAA GUC GUUUGGC
546	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
549	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
598	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
603	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
631	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
634	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
675	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
691	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
764	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
803	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
895	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
906	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
920	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
953	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
1175	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
1220	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
1230	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
1256	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU
1274	GUUUAAGU AGAA GGUUCC ACCAGAGAAACACAGUGUGUGUACAUUACUUGUA	GUUUGG GUC GUUUGGU

1393	UGUCUGAA	AGAA	GCUUC	ACCAGAGAAACACACAGGUGUGUGURCAUUAUACUGUA	GGAGCU	GUC	UUCAGACA
1435	CAGGUGG	AGAA	GCUCAG	ACCAGAGAAACACACAGGUGUGUGURCAUUAUACUGUA	CUAGCU	GUC	CCACCUU
1525	GUACACCA	AGAA	GGGUA	ACCAGAGAAACACACAGGUGUGUGURCAUUAUACUGUA	UAACCU	GAU	UUGGUGAC
1542	GAUGAGCC	AGAA	GGGUG	ACCAGAGAAACACACAGGUGUGUGURCAUUAUACUGUA	CCAGCU	GUC	GUACAUUC
1564	CCGUGGG	AGAA	GGGUU	ACCAGAGAAACACACAGGUGUGUGURCAUUAUACUGUA	AAUUCU	GUU	CCACAGG

Table 29: Human bcr/abl HH Target Sequence

Sequence ID No.	HH Target Sequence
<u>b2-a2</u> <u>Junction</u>	
20	UGGCCAACA AUA AGGAGAGAGOC
21	GAAGAGOC CUU CAGGGGOCAGU
22	AAGAGGOC UUC AGGGGOCAGUA
<u>b3-a2</u> <u>Junction</u>	
23	UAAGCAGAG UUC AAAAGGOCUUC
24	UCAAAGOC CUU CAGGGGOCAGU
25	CAAAAGOC UUC AGGGGOCAGUA

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Table 30: Human *bcr-abl* HH Ribozyme Sequences

Sequence ID No.	HH Ribozyme Sequence
26	GCCUUCUUCU CUGAUGAGGCGGAAGGCGGA AUGAUGGUC
27	ACUGGCGCG CUGAUGAGGCGGAAGGCGGA AGGCUUCUC
28	UACGCGCGCU CUGAUGAGGCGGAAGGCGGA AAGGCUUCU
29	GAAGGCUUUU CUGAUGAGGCGGAAGGCGGA AACUCUGCUA
30	ACUGGCGCG CUGAUGAGGCGGAAGGCGGA AGGCUUUUG
31	UACUGGCGCU CUGAUGAGGCGGAAGGCGGA AAGGCUUUU

Table 31: RSV (1B) HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
10	GGCAAAU A AADCAAU	276	AAAADAU A CUGAADA
14	AAUAAAU C AADUCAG	283	ACUGAAU A CAACACA
18	AAUCAAU U CAGOCAA	295	ACAAAAU A UGGCACU
19	ADCAAUU C AGOCAAC	303	UGGCACU U UCCCTAU
54	CAADGAU A AUACACC	304	GGCACUU U CCGUADG
57	UGAUAU A CACACCA	305	GCACUUU C CCGAUGC
77	UGAUGAU C ACAGACA	309	UUUCCCU A UGOCAAU
94	AGACCGU U GUCACUU	317	UGOCAAU A UUCADCA
97	CCGUUGU C ACUUGAG	319	CCAADAU U CAUCAAU
101	UGUCACU U GAGACCA	320	CAADAUU C AUCAADU
110	AGACCAU A AUACAU	323	UAUOCAU C AADCAUG
113	CCAUAAU A ACAUCAC	327	CAUCAU C AUGAUGG
118	AUAACAU C ACUAAAC	337	GAUGGGU U CUUAGAA
122	CAUCACU A ACCAGAG	338	ADGGGUU C UUAAGAU
134	GAGACAU C AUAACAC	340	GGGUUCU U AGAUGC
137	ACAUCAU A ACACACA	341	GGUUCUU A GAUUGCA
148	CACAAAU U UAUAUAC	350	AAUGCAU U GGCATUA
149	ACAAAUU U AUUAACU	356	UUGGCAU U AAGOCUA
150	CAAAUUU A UAUAUUU	357	UGGCAUU A AGOCUAC
152	AAUUAU A UACUUGA	363	UAAGOCU A CAAAGCA
154	UUUAUAU A CUUGAUA	372	AAAGCAU A CUCCCAU
157	AUAUAU U GAUAUAU	375	GCAUAU C CCUAUAU
161	ACUUGAU A AAUCAUG	380	CUCCCAU A AUUAACA
165	GAUAAAU C AUGAADG	383	CCAUAAU A UACAAGU
176	AAUGCAU A GUGAGAA	385	AUAUAU A CAAGUAU
188	GAAACU U GAUGAAA	391	UACAAGU A UGAUCCU
208	GOCACAU U UACAUUC	396	GUADGAU C UCAAUCC
209	CCACAUU U ACAUCCU	398	AUGAUUU C AADUCAU
210	CACAUUU A CAUCCUU	402	UCUCAU C CAUAAAU
214	UUUACAU U CCUGGUC	406	AAUGCAU A AAUUUCA
215	UUACAUU C CUGGUCU	410	CAUAAU U UCAACAC
221	UCCUGGU C AACUAUG	411	AUAUAU U CAACACA
226	GUCACU A UGAAADG	412	UAAAUUU C AACACAA
239	UGAAACU A UUAACAA	421	ACACAAU A UUCACAC
241	AAAUUAU U ACACAAA	423	ACAAUAU U CACACAA
242	AAAUUAU A CACAAAG	424	CAAUUAU C ACACAAU
251	ACAAAGU A GGAAGCA	432	ACACAAU C UAAAACA
261	AAGCACU A AAUAUAU	434	ACAAUUA A AAACAAC
265	ACUAAAU A UAAAAA	446	AACACU C UAUGCAU
267	UAAAUUA A AAAAUA	448	CAACUU A UGCAUAA
274	AAAAAU A UACUGAA	454	UAUGCAU A ACUAUAC

458	CAIRACU A UACUCCA
460	UAACIAU A CUCCADA
463	CUAIAU C CAIAGUC
467	ACUCCAU A GUCCAGA
470	CCAIAGU C CAGAUCC
489	UGAAAAU U AUAAGUA
490	GAAAAU A UAGUAU
492	AAAUUAU A GUAAUU
495	UAUAAGU A AUUAUA

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Table 32: RSV (1B) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
10	AUUGAUU CUGAUGAGGCOGAAAGGCOGAA AUUGOC
14	CUGAUUAU CUGAUGAGGCOGAAAGGCOGAA AUUUAUU
18	UUGGCTG CUGAUGAGGCOGAAAGGCOGAA AUUGAUU
19	GUUGGCU CUGAUGAGGCOGAAAGGCOGAA AAUUGAU
54	GGUGUAU CUGAUGAGGCOGAAAGGCOGAA AUCAUUG
57	UGUGGUG CUGAUGAGGCOGAAAGGCOGAA AUUAUCA
77	UGUCUGU CUGAUGAGGCOGAAAGGCOGAA AUCAUCA
94	AAGUGAC CUGAUGAGGCOGAAAGGCOGAA ACGGUCU
97	CUCAAGU CUGAUGAGGCOGAAAGGCOGAA ACAACGG
101	UGGUCUC CUGAUGAGGCOGAAAGGCOGAA AGUGACA
110	AUGUUAU CUGAUGAGGCOGAAAGGCOGAA AUGGUCU
113	GUGAUGU CUGAUGAGGCOGAAAGGCOGAA AUUAUGG
118	GGUUAU CUGAUGAGGCOGAAAGGCOGAA AUGUUAU
122	CUUGGU CUGAUGAGGCOGAAAGGCOGAA AGUGAUG
134	GUGUUAU CUGAUGAGGCOGAAAGGCOGAA AUGUCUC
137	UGUGUGU CUGAUGAGGCOGAAAGGCOGAA AUGAUGU
148	GUUAUAU CUGAUGAGGCOGAAAGGCOGAA AUUGUG
149	AGUAUAU CUGAUGAGGCOGAAAGGCOGAA AAUUGU
150	AAGUAUA CUGAUGAGGCOGAAAGGCOGAA AAUUG
152	UCAAGUA CUGAUGAGGCOGAAAGGCOGAA AUAAAUU
154	UAUCAAG CUGAUGAGGCOGAAAGGCOGAA AUUAUA
157	AUUUAUC CUGAUGAGGCOGAAAGGCOGAA AGUAUAU
161	CAUGAUU CUGAUGAGGCOGAAAGGCOGAA AUCAAGU
165	CAUUAU CUGAUGAGGCOGAAAGGCOGAA AUUAUC
176	UUCUCAC CUGAUGAGGCOGAAAGGCOGAA AUGCAUU
188	UUUCAUC CUGAUGAGGCOGAAAGGCOGAA AGUUUUC
208	GAUGUA CUGAUGAGGCOGAAAGGCOGAA AUGGGC
209	GGAAUGU CUGAUGAGGCOGAAAGGCOGAA AAUGUGG
210	AGGAUG CUGAUGAGGCOGAAAGGCOGAA AAUGUG
214	GACCAAG CUGAUGAGGCOGAAAGGCOGAA AUGUAA
215	UGACCAAG CUGAUGAGGCOGAAAGGCOGAA AAUGUA
221	CAUAGUU CUGAUGAGGCOGAAAGGCOGAA ACCAGGA
226	CAUUAU CUGAUGAGGCOGAAAGGCOGAA AGUUGAC
239	UGUGUA CUGAUGAGGCOGAAAGGCOGAA AGUUUA
241	UUUGUGU CUGAUGAGGCOGAAAGGCOGAA AUAGUUU
242	CUUGUG CUGAUGAGGCOGAAAGGCOGAA AAUAGUU
251	UGUUUC CUGAUGAGGCOGAAAGGCOGAA ACUUUGU
261	UUUAUAU CUGAUGAGGCOGAAAGGCOGAA AGUGCUU
265	UUUUUA CUGAUGAGGCOGAAAGGCOGAA AUUAAGU
267	UAUUUU CUGAUGAGGCOGAAAGGCOGAA AUUAUA
274	UUCAGUA CUGAUGAGGCOGAAAGGCOGAA AUUUUU
276	UAUUCAG CUGAUGAGGCOGAAAGGCOGAA AUUAUUU

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283	UGUGUUG	CUGAUGAGGCOGAAAGGCOGAA	ADUCAGU
295	AGUGCCA	CUGAUGAGGCOGAAAGGCOGAA	ADUUGU
303	AUAGGGA	CUGAUGAGGCOGAAAGGCOGAA	AGUGCCA
304	CADAGGG	CUGAUGAGGCOGAAAGGCOGAA	AAGUGCC
305	GCAUAGG	CUGAUGAGGCOGAAAGGCOGAA	AAAGUGC
309	AUUGGCA	CUGAUGAGGCOGAAAGGCOGAA	AGGGAUA
317	UGAUGAA	CUGAUGAGGCOGAAAGGCOGAA	ADUGGCA
319	AUUGAUG	CUGAUGAGGCOGAAAGGCOGAA	AUAUUGG
320	GADUGAU	CUGAUGAGGCOGAAAGGCOGAA	AUAUUGG
323	CADGAU	CUGAUGAGGCOGAAAGGCOGAA	AUGAUA
327	CCAUCAU	CUGAUGAGGCOGAAAGGCOGAA	ADUGAUG
337	UUCUAG	CUGAUGAGGCOGAAAGGCOGAA	ACCCADC
338	ADUCCA	CUGAUGAGGCOGAAAGGCOGAA	AAUCCA
340	GCAUUCU	CUGAUGAGGCOGAAAGGCOGAA	AGAAUCC
341	UGCAUUC	CUGAUGAGGCOGAAAGGCOGAA	AGAAUCC
350	UAUUGCC	CUGAUGAGGCOGAAAGGCOGAA	ADUGCAU
356	UAGGCUU	CUGAUGAGGCOGAAAGGCOGAA	ADUGCAA
357	GUAGGCU	CUGAUGAGGCOGAAAGGCOGAA	ADUGCAA
363	UGCUUUG	CUGAUGAGGCOGAAAGGCOGAA	AGGCUUA
372	ADGGGAG	CUGAUGAGGCOGAAAGGCOGAA	ADGCUU
375	AUUAUGG	CUGAUGAGGCOGAAAGGCOGAA	AGUAUUG
380	UGUAUUA	CUGAUGAGGCOGAAAGGCOGAA	AUGGGAG
383	ACUUGUA	CUGAUGAGGCOGAAAGGCOGAA	ADUBUGG
385	AUAUUG	CUGAUGAGGCOGAAAGGCOGAA	AUAUUAU
391	GAGAUCA	CUGAUGAGGCOGAAAGGCOGAA	ACUUGUA
396	GGAUUGA	CUGAUGAGGCOGAAAGGCOGAA	ADCAUAC
398	AUGGAU	CUGAUGAGGCOGAAAGGCOGAA	AGAUCAU
402	AUUAUG	CUGAUGAGGCOGAAAGGCOGAA	ADUGAGA
406	UGAAAU	CUGAUGAGGCOGAAAGGCOGAA	ADGGAU
410	GUGUUGA	CUGAUGAGGCOGAAAGGCOGAA	ADUUAUG
411	UGUGUUG	CUGAUGAGGCOGAAAGGCOGAA	AAUUAU
412	UUGUGUU	CUGAUGAGGCOGAAAGGCOGAA	AAUUAU
421	GUGUGAA	CUGAUGAGGCOGAAAGGCOGAA	ADUGUGU
423	UUGUGUG	CUGAUGAGGCOGAAAGGCOGAA	AUAUUGU
424	ADUGUGU	CUGAUGAGGCOGAAAGGCOGAA	AAUUAUG
432	UGUUUUA	CUGAUGAGGCOGAAAGGCOGAA	ADUGUGU
434	GUUGUUU	CUGAUGAGGCOGAAAGGCOGAA	AGAUUGU
446	AUGCAUA	CUGAUGAGGCOGAAAGGCOGAA	AGUUGUU
448	UUAUGCA	CUGAUGAGGCOGAAAGGCOGAA	AGAGUUG
454	GUUAUGU	CUGAUGAGGCOGAAAGGCOGAA	ADUGCAU
458	UGGAGUA	CUGAUGAGGCOGAAAGGCOGAA	AGUAUUG
460	UAUGGAG	CUGAUGAGGCOGAAAGGCOGAA	ADUGUAU
463	GACUAUG	CUGAUGAGGCOGAAAGGCOGAA	AGUAUUG
467	UCUGGAC	CUGAUGAGGCOGAAAGGCOGAA	ADUGGAGU
470	CCAUUCG	CUGAUGAGGCOGAAAGGCOGAA	ACUAUUG
489	UUAUUAU	CUGAUGAGGCOGAAAGGCOGAA	ADUUAUA
490	AUAUUAU	CUGAUGAGGCOGAAAGGCOGAA	ADUUAUA
492	AAAUUAU	CUGAUGAGGCOGAAAGGCOGAA	ADUUAUA
495	UUUAAAU	CUGAUGAGGCOGAAAGGCOGAA	ACUAUUA

Table 33 : RSV (1C) HH target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
10	GGCAAU A AGAUAU	165	UACAUU A ACTAACG
16	UAAGAU U UGAUAG	169	UUUACU A ACGCUU
17	AAGAUAU U GAUAAGU	175	UAACGU U UGGCUA
21	AUUGAU A AGUACCA	176	AACGUU U GGUUAG
25	GAUAAGU A CCACUA	181	UUUGGU A AGGCAU
31	UACCAU U AAUUUA	192	CAGUAU A CAUCAA
32	ACCACU A AAUUUA	196	GUAUAU A CAUCAA
36	CUUAAU U UAACUC	201	AUACAU C AAUUUA
37	UUAAAU U AACUCC	206	AUCAAU U GAUUGG
38	UAAAUU A ACUCCU	216	AUGGCU U GUGUUG
42	UUUACU C CCUUGU	221	AUUGGU U UGUCAU
46	ACUCCU U GGUAGA	222	UUGGUU U GUGUUG
50	CCUUGU U AGAGUG	231	UGCAU U AUUACA
51	CUUGU A GAGUGG	232	GCAU U UUAAG
67	CAGCAU U CAUUGG	234	AUGUAU U ACAAGU
68	AGCAU C AUUGU	235	UGUAU A CAUGG
71	AUUAU U GAGUAG	241	UACAAGU A GUGUAU
76	AUGAGU A UGAUAA	247	UAGUAU A UUGUCC
81	GUAGAU A AAAGUA	249	GUGUAU U UGUUUA
87	UAAAGU U AGAUAC	250	UGUAU U GUUUA
88	AAAAGU A GAUACA	256	UUGUCC A AUUAUA
92	GUAGAU U ACAAAU	259	CCUAU A AUUAUA
93	UUAUAU A CAAAUU	262	UAUAU A AUUAU
100	ACAAAU U UGUUGA	265	UAUAU A UGUUGU
101	CAAAAU U GUUGAC	267	AUAUAU U GUUGUA
104	AUUGU U UGACAU	270	AUAUGU A GUAAAU
105	AUUGU U GACAU	273	UUGUAU A AAUCCA
120	AUGAGU A GCAUGU	278	GUAAAU C CAUUC
125	GUAGAU U GUUAAA	283	AUCCAU U UCACAAC
128	GCAUGU U AAAAUA	284	UCCAU U CACAACA
129	CAUUGU A AAAUA	285	CCAUU C ACAACA
135	UAAAU A ACUGGU	300	UGGAGU A CUACAA
143	ACUGGU A UACUGU	303	CAGUAU A CAAAU
145	AUGUAU A CUGUA	316	UGGAGU U AUUAUG
151	UACUGU A AUUAU	317	GGAGGU A AUUAUG
155	GAUAAU U AUUAU	319	AGGUUA A UAUUGG
156	AUAUAU A AUUAU	321	GUUAUA A UGGGAA
159	AUUAU A CAUUA	338	AUGGAU U AACCAU
163	AUUAU U UAACUA	339	UGGAU A ACACAU
164	AUUAU U AACUA	346	AACCAU U GUUUA

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350 CAUUGCU C UCAACCU
352 UUGCUU C AACCUA
358 UCAACCU A AUGGCU
364 UAUUGCU C UACUAGA
366 AUGGCU A CUAGAG
369 GUUACU A GAUGACA
379 UGACAAU U GUGAAAU
387 GUGAAAU U AAADUCU
388 UGAAAU A AAUUCU
392 AUUAAU U CUUCAA
393 UUAUUU C UCUAAA
395 AAADUCU C CAAAAA
405 AAAACU A AGUGAU
412 AAGUGAU U CAACAU
413 AGUGAU C AACUAG
427 GACCAU U AUUAGA
428 ACCAAU A UAUAGU
430 CAUUU A UGAUCA
436 UAUAGU C AAUUAU
440 AUUUAU U AUUUAU
441 AUUUAU A UCUUUA
443 CAUUU C UGAUUA
449 UCUUUA U ACUUGA
450 CUUUAU A CUUGAU
453 AUUUAU U GUUUUG
458 CUUGAU U UGUAU
459 UUGUAU U GAUUA
463 AUUUAU C UUAUUC
465 UGUUAU U AAUUAU
466 UGUUAU A AUUUAU
469 UCUUUA C CAUUAU
473 AUUUAU A AAUUAU
477 CAUUAU U AUUUAU
478 AUUUAU A UUAUUA
480 AAUUAU A AUUUAU
483 UUAUUA U AAUUAU
484 UUAUUA A AUUUAU
487 AUUUAU A UCAUUA
489 UUAUUA C AAUUAU
494 AUUUAU A GUAUUA
501 AGUUAU C AAUUAU
507 UCAUUA C AUUUAU
511 UGUUAU A ACUUAU
519 ACUUAU U AGUUAU
520 CUUUAU A GUUUAU
523 CAUUAU U AUUUAU
524 AUUUAU A AUUUAU

Table 34: RSV (1C) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
10	AAAUUCU CUGAUGAGGCGGAAAGGCGGAA AUUUGGC
16	CUUADCA CUGAUGAGGCGGAAAGGCGGAA AUUUCUA
17	ACUUAAC CUGAUGAGGCGGAAAGGCGGAA AAUUCUU
21	UGGUACU CUGAUGAGGCGGAAAGGCGGAA AUCAAUU
25	UAAGUGG CUGAUGAGGCGGAAAGGCGGAA ACUUAAC
31	UAAAUUU CUGAUGAGGCGGAAAGGCGGAA AGUGGUA
32	UUAAAUU CUGAUGAGGCGGAAAGGCGGAA AAGUGGU
36	GGAGUUA CUGAUGAGGCGGAAAGGCGGAA AUUUAAG
37	GGGAGUU CUGAUGAGGCGGAAAGGCGGAA AAUUAUA
38	AGGGAGU CUGAUGAGGCGGAAAGGCGGAA AAAUUUA
42	ACCAAGG CUGAUGAGGCGGAAAGGCGGAA AGUUAUA
46	UCUAACC CUGAUGAGGCGGAAAGGCGGAA AGGGAGU
50	CAUCUCU CUGAUGAGGCGGAAAGGCGGAA ACCAAGG
51	CCAUUCU CUGAUGAGGCGGAAAGGCGGAA AACCAAG
67	CUCAADG CUGAUGAGGCGGAAAGGCGGAA AUUGGCG
68	ACUCAAU CUGAUGAGGCGGAAAGGCGGAA AAGUGGU
71	CAUACUC CUGAUGAGGCGGAAAGGCGGAA AUGAAUU
76	UUUADCA CUGAUGAGGCGGAAAGGCGGAA ACUCAAU
81	UAACUUU CUGAUGAGGCGGAAAGGCGGAA AUCAUAC
87	GUAAUCU CUGAUGAGGCGGAAAGGCGGAA ACUUAUA
88	UGUAUUC CUGAUGAGGCGGAAAGGCGGAA AACUUUU
92	AUUUUGU CUGAUGAGGCGGAAAGGCGGAA AUUUAAC
93	AAUUGUG CUGAUGAGGCGGAAAGGCGGAA AAUUAUA
100	UCAAACA CUGAUGAGGCGGAAAGGCGGAA AUUUUGU
101	GUCAAAC CUGAUGAGGCGGAAAGGCGGAA AAUUUUG
104	AUUGUCA CUGAUGAGGCGGAAAGGCGGAA ACUAAUU
105	CAUUGUC CUGAUGAGGCGGAAAGGCGGAA AACUAAU
120	ACAADGC CUGAUGAGGCGGAAAGGCGGAA ACUUAUU
125	UUUUAAC CUGAUGAGGCGGAAAGGCGGAA AUGCUAC
128	UAUUUUU CUGAUGAGGCGGAAAGGCGGAA ACAUUGC
129	UUAUUUU CUGAUGAGGCGGAAAGGCGGAA AACAAUG
135	AGCAUGU CUGAUGAGGCGGAAAGGCGGAA AUUUUUA
143	AUCAGUA CUGAUGAGGCGGAAAGGCGGAA AGCAUGU
145	UUADUAG CUGAUGAGGCGGAAAGGCGGAA AUAGCAU
151	AUUAUUU CUGAUGAGGCGGAAAGGCGGAA AUCAGUA
155	AUGUAUU CUGAUGAGGCGGAAAGGCGGAA AUUUAUC
156	AAUGUAU CUGAUGAGGCGGAAAGGCGGAA AAUUUAU
159	UUAAAUU CUGAUGAGGCGGAAAGGCGGAA AUUAUUU
163	UUAGUUA CUGAUGAGGCGGAAAGGCGGAA AUGUAUU
164	GUUAGUU CUGAUGAGGCGGAAAGGCGGAA AAUGUAU
165	CGUUAUU CUGAUGAGGCGGAAAGGCGGAA AAUUAUA

169	AAAGGCU	CUGAUGAGGCGAAAGGCGGA	AGUUA
175	UUAGCCA	CUGAUGAGGCGAAAGGCGGA	AGGCUA
176	CUUAGCC	CUGAUGAGGCGAAAGGCGGA	AAGGCU
181	ACUGCCU	CUGAUGAGGCGAAAGGCGGA	AGCCAA
192	UUGUAUG	CUGAUGAGGCGAAAGGCGGA	AUCACU
196	UUGAUUG	CUGAUGAGGCGAAAGGCGGA	AUGUAC
201	UCAAUUU	CUGAUGAGGCGAAAGGCGGA	AUUGUA
206	GCCAUUC	CUGAUGAGGCGAAAGGCGGA	AUUGAU
216	CAACAC	CUGAUGAGGCGAAAGGCGGA	AUGCCAU
221	AUGACA	CUGAUGAGGCGAAAGGCGGA	ACACAU
222	CAUGCA	CUGAUGAGGCGAAAGGCGGA	AACACA
231	UUGUAU	CUGAUGAGGCGAAAGGCGGA	ACAUCA
232	CUUGUA	CUGAUGAGGCGAAAGGCGGA	AACAGC
234	UACUUG	CUGAUGAGGCGAAAGGCGGA	AUAACU
235	CUACUUG	CUGAUGAGGCGAAAGGCGGA	AUAACA
241	AUAUAC	CUGAUGAGGCGAAAGGCGGA	ACUUGA
247	GGGCAA	CUGAUGAGGCGAAAGGCGGA	AUCACU
249	UAGGGCA	CUGAUGAGGCGAAAGGCGGA	AUAUAC
250	UAGGGC	CUGAUGAGGCGAAAGGCGGA	AUAUCA
256	UUAUUU	CUGAUGAGGCGAAAGGCGGA	AGGCAA
259	AUAUUU	CUGAUGAGGCGAAAGGCGGA	AUUGGG
262	ACAUUU	CUGAUGAGGCGAAAGGCGGA	AUUAUA
265	ACUACA	CUGAUGAGGCGAAAGGCGGA	AUUAUA
267	UUAUAC	CUGAUGAGGCGAAAGGCGGA	AUAUAU
270	AUUUAC	CUGAUGAGGCGAAAGGCGGA	ACAUUA
273	UGGAUU	CUGAUGAGGCGAAAGGCGGA	ACUACA
278	GAAUUG	CUGAUGAGGCGAAAGGCGGA	AUUUAC
283	GUUGUA	CUGAUGAGGCGAAAGGCGGA	AUUGAU
284	UGUUGU	CUGAUGAGGCGAAAGGCGGA	AUUGGA
285	UUGUUG	CUGAUGAGGCGAAAGGCGGA	AAUUGG
300	UUUGUAG	CUGAUGAGGCGAAAGGCGGA	ACUGCA
303	CAUUUG	CUGAUGAGGCGAAAGGCGGA	AGUACU
316	CAUAUA	CUGAUGAGGCGAAAGGCGGA	ACCUCA
317	CCAUUA	CUGAUGAGGCGAAAGGCGGA	AACCUU
319	UCCAUU	CUGAUGAGGCGAAAGGCGGA	AUAACU
321	UUUCCA	CUGAUGAGGCGAAAGGCGGA	AUAUAU
338	ADGUGU	CUGAUGAGGCGAAAGGCGGA	AUUCAU
339	AADUGU	CUGAUGAGGCGAAAGGCGGA	AAUCCA
346	UGAGAGC	CUGAUGAGGCGAAAGGCGGA	ADGUGU
350	AGGUUGA	CUGAUGAGGCGAAAGGCGGA	AGCAUG
352	UAAGGUU	CUGAUGAGGCGAAAGGCGGA	AGAGCA
358	AGACAU	CUGAUGAGGCGAAAGGCGGA	AGGUUGA
364	UCUAGUA	CUGAUGAGGCGAAAGGCGGA	ACCAUA
366	CAUCUAG	CUGAUGAGGCGAAAGGCGGA	AGACAU
369	UGUACU	CUGAUGAGGCGAAAGGCGGA	AGUAGC
379	AUUUAC	CUGAUGAGGCGAAAGGCGGA	AUUGUA
387	AGAUUU	CUGAUGAGGCGAAAGGCGGA	AUUUAC
388	GAGAUU	CUGAUGAGGCGAAAGGCGGA	AAUUCA
392	UUUGGAG	CUGAUGAGGCGAAAGGCGGA	AUUUAU

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393	UUUUGGA	CUGAUGAGGCGGAAAGGCGCGAA	AAUUUAA
395	UUUUUUG	CUGAUGAGGCGGAAAGGCGCGAA	AGAUUUU
405	AAUCACU	CUGAUGAGGCGGAAAGGCGCGAA	AGUUUUU
412	AUUGUUU	CUGAUGAGGCGGAAAGGCGCGAA	AUCACUU
413	CAUUGUU	CUGAUGAGGCGGAAAGGCGCGAA	AADCACU
427	UUCAUUU	CUGAUGAGGCGGAAAGGCGCGAA	AUUGGUC
428	AUUCAUU	CUGAUGAGGCGGAAAGGCGCGAA	AAUUGGU
430	UGAUUCA	CUGAUGAGGCGGAAAGGCGCGAA	AUAUUUG
436	GAAUAUU	CUGAUGAGGCGGAAAGGCGCGAA	AUUCAUU
440	UUCAGAU	CUGAUGAGGCGGAAAGGCGCGAA	AUUGAUU
441	AUUCAGA	CUGAUGAGGCGGAAAGGCGCGAA	AAUUGAU
443	UAADUCA	CUGAUGAGGCGGAAAGGCGCGAA	AUAUUUG
449	UCCAGAU	CUGAUGAGGCGGAAAGGCGCGAA	AUUCAGA
450	AUCCAGG	CUGAUGAGGCGGAAAGGCGCGAA	AAUUCAG
453	CAAUUC	CUGAUGAGGCGGAAAGGCGCGAA	AGUAUUU
458	AAGADCA	CUGAUGAGGCGGAAAGGCGCGAA	AUCCAGG
459	UAAGAUU	CUGAUGAGGCGGAAAGGCGCGAA	AAUCCAA
463	GGAUUA	CUGAUGAGGCGGAAAGGCGCGAA	AUCAAUU
465	AUGGAUU	CUGAUGAGGCGGAAAGGCGCGAA	AGAUCAA
466	UAUGGAU	CUGAUGAGGCGGAAAGGCGCGAA	AAGAUCA
469	AUUUAUG	CUGAUGAGGCGGAAAGGCGCGAA	AUUAAGA
473	UAUAUUU	CUGAUGAGGCGGAAAGGCGCGAA	AUGGAUU
477	UAUUUAU	CUGAUGAGGCGGAAAGGCGCGAA	AUUUAUG
478	UUUAUUU	CUGAUGAGGCGGAAAGGCGCGAA	AAUUUUU
480	UAUUAAU	CUGAUGAGGCGGAAAGGCGCGAA	AUAUUUU
483	UGAUUUU	CUGAUGAGGCGGAAAGGCGCGAA	AUUUUAA
484	UUGAUUU	CUGAUGAGGCGGAAAGGCGCGAA	AAUUUAU
487	UAUUUGA	CUGAUGAGGCGGAAAGGCGCGAA	AUUUAUU
489	GUUUAUU	CUGAUGAGGCGGAAAGGCGCGAA	AUAUUUA
494	GAUUUUC	CUGAUGAGGCGGAAAGGCGCGAA	AGUUUAU
501	UGACAUU	CUGAUGAGGCGGAAAGGCGCGAA	AUUUGCU
507	UGUUAGU	CUGAUGAGGCGGAAAGGCGCGAA	ACAUUGA
511	AUGGUGU	CUGAUGAGGCGGAAAGGCGCGAA	AGUGACA
519	AUUUAUU	CUGAUGAGGCGGAAAGGCGCGAA	AUGGUGU
520	UAUUUAC	CUGAUGAGGCGGAAAGGCGCGAA	AAUGGUG
523	UUUAUUU	CUGAUGAGGCGGAAAGGCGCGAA	AAUUUUU
524	UUUAUUU	CUGAUGAGGCGGAAAGGCGCGAA	AAUUUUU

Table 35: RSV (N) HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
9	GGCAAAU A CAAAGAU	217	GGUADGU U AUADGGG
21	GAUGGUU C UUAGCAA	218	GUADGUU A UADGGGA
23	UGGCUU U AGCAAAG	220	AUGUUAU A UGCGAUG
24	GGCUUU A GCAAAGU	229	GGUADGU C UAGGUUA
32	GCAAAGU C AAGUUGA	231	GAUGGUU A GGUUAGG
37	GUCAAGU U GAUGAUU	235	UCUAGGU U AGGAAGA
45	GAUGAUU A CACUCAA	236	CUAGGUU A GGAAGAG
50	AUACAUU C AACAAAG	254	ACACCAU A AAAAUAC
60	CAAAGAU C AACUUUU	260	UAAAAAU A CUUAGAG
65	AUCAAUU U CUGUCAU	263	AAAUACU C AGGAGUG
66	UCAAUUU C UGUCAUC	277	GUGGUUU A UCAUGUA
70	CUUCUGU C AUCCAGC	279	GGUUAUU C AUGUAAA
73	CUGUCAU C CAGCAA	284	AUCAUGU A AAGCAA
82	AGCAAAU A CACCAUC	299	AUGGAGU A GUUGUAA
89	ACACCAU C CAAAGGA	305	UAGAUGU A ACAACAC
108	AGGAGAU A GUADUGA	315	AACACAU C GUCAAGA
111	AGAUAGU A UUGAUAC	318	ACAUUGU C AAGACAU
113	AUAGUAU U GAUACUC	326	AAGACAU U AAUGGAA
117	UAUUGAU A CUUUUAA	327	AGACAUU A AUGGAAA
120	UGAUACU C CUAAUUA	346	AUGAAAU U UGAAGUG
123	UACUCCU A AUUADGA	347	UGAAAUU U GAAGUGU
126	UCCUAAU U AUGADGU	355	GAAGUGU U AACAUUG
127	CCUAAAU A UGADGUG	356	AAGUGUU A ACAUUGG
146	AACACAU C AAUAAGU	361	UUAACAU U GCGAAGC
150	CAUCAAU A AGUUAUG	370	GCAAGCU U AACAAAU
154	AAUAAGU U AUGUGGC	371	CAAGCUU A ACAACUG
155	AUAAGUU A UGUGGCA	383	CUGAAAU U CAUAUCA
166	GCAUGU U AUUAADC	384	UGAAAUU C AAADCAA
167	GCAUGUU A UUAADCA	389	UUCAAAU C AACAUUG
169	AUGUUAU U AAUCACA	395	UCAACAU U GAGUAG
170	UGUUAUU A AUCACAG	401	UUGAGAU A GAUUAUA
173	UAUUAUU C ACAGAAG	406	AUAAGAU C UAGAAA
186	AGAUUGU A AUCAUAA	408	AGAAUUC A GAAAUUC
189	UGCUAAU C AUAAAUU	415	AGAAAAU C CUACAAA
192	UAADCAU A AAUOCAC	418	AAAUCCU A CAAAAA
196	CAUAAAU U CACUGGG	431	AAAUCCU A AAAGAAA
197	AUAAAUU C ACUGGGU	449	GAGAGGU A GUUCCAG
205	ACUGGGU U AAUAGGU	453	GGUAGCU C CAGAAUA
206	CUUGGUU A AUAGGUA	460	CCAGAAU A CAGGCAU
209	GGUUAUU A GGUADGU	472	CAUGACU C UCCUGAU
213	AAUAGGU A UGUUAUA	474	UGACUCU C CUGAUUG

480	UCCUGAU U GUGGGAU	696	UUUUGGU A UAGCACA
491	GGADGAU A AUAUUAU	698	UUGGUU A GCACAAU
494	UGAUAU A UUAUGUA	706	GCACAAU C UUCUACC
496	AUAUAU U AUGUAUA	708	ACUAUCU U CUACCAG
497	UAUAUAU A UGUADAG	709	CAUUCU C UACCAGA
501	AUAUAGU A UAGCAGC	711	ADCUUCU A CCAGAGG
503	UADGUU A GCAGCAU	726	UGGCAGU A GAGUUGA
511	GCAGCAU U AGUAUA	731	GUAGAGU U GAAGGGA
512	CAGCAU A GUAAUA	740	AAGGGAU U UUGCAG
515	CAUAGU A AUAUA	741	AGGGAU U UUGCAGG
518	UAGUAU A AUAUAU	742	GGGAU U UGCAGGA
522	AUAUAU A AUAUAGC	743	GGAUU U GCAGCAU
526	ACUAUAU U AGCAGCA	751	GCAGCAU U GUUAUG
527	CUAUAU A GCAGCAG	754	GGAUUGU U UADGAU
544	GACAGU C UGGUCU	755	GADUGU U AUGAUG
549	ADUGGU C UACAGC	756	ADUGUU A UGADGC
551	CUGGUCU U ACAGCUG	766	AADGCU A UGGUGCA
552	UGGUCU A CAGCUG	787	GUGAGU U ACGGUGG
563	CCUGAU U AGGAGAG	788	UGAGU A CGGUGG
564	CCUGAU A GGAGAGC	800	GGGAGU C UAGCAA
573	GAGAGU A AUAUGU	802	GGAGU U AGCAAA
576	AGCUAU A AUGUCCU	803	GGAGU A GCAAAU
581	AUAUGU C CUAAAA	811	GCAAAU C AGUUA
584	AUGUCCU A AAAAAUG	815	AADCAU U AAAAA
603	GAAAGU U ACAAAGG	816	AUCAGU A AAAUAU
604	AAAGGU A CAAGGC	822	UAAAAU A UUAUGU
613	AAAGGU U ACUACCC	824	AAAAU U AUGUAG
614	AAGGU A CUACCA	825	AAUAU A UGUUAGG
617	GUUAU A CCAAGG	829	AUAUGU U AGGACU
629	AGGACU A GCAACA	830	UUAUGU A GGCAGG
640	AACAGU U CUAGAA	840	ACAGGU A GUGUGCA
641	ACAGGU C UAUGAAG	866	AACAAGU U GUUGAGG
643	AGGUU A UGAAGG	869	AAGUGU U GAGGUU
652	GAAGGU U UGAAAA	875	UUGAGU U UADGAU
653	AAGGUU U GAAAAAC	876	UGAGGU U AUGAUA
663	AAACAU C CCAACU	877	GAGGUU A UGAUAU
670	CCCAU U UAUAU	883	UAUAU A UGOCOA
671	CCCAU U AUAAGG	895	CAAAAU U GGGUGU
672	CCCAU A UAGAGU	913	GCAGGU U CUACCAU
674	ACUUAU A GAUGUU	914	CAGGU C UACCAU
680	UAGAGU U UUGUUC	916	GGAUU A CCAUAU
681	AGAUGU U UUGUUA	921	CUACCAU A UAUGAA
682	GAUGUU U UGUUUA	923	ACCAUAU A UUGAACA
683	AUGUUU U GUUUAU	925	CAUAUAU U GAACAAC
686	UUUUGU U CAUUGG	943	AAAGCAU C AUAUAU
687	UUUGUU C AUUUGG	946	GCAUCAU U AUUAUCU
690	UGUUAU U UUGGUU	947	CAUCAU A UAUCU
691	GUUUAU U UGUUAU	949	UCAUAU U AUUUG
692	UUAUAU U GGUUAU	950	CAUAUAU A UCUUGA

952	UUUUUU C UUUGACU
954	AUUUUU U UGACUCA
955	UUUUUU U GACUCAA
960	UUUGACU C AAUUUCC
964	ACUCAAU U UCCUCCAC
965	CUCAAUU U CCUCCAU
966	UCAUUU C CUCCAUU
969	AUUUUU C ACUUUUC
973	CCUCCAU U CUCCAGU
974	CUCCAUU C UCCAGUG
976	CACUUU C CAGUGUA
983	CCAGUGU A GUUUUAG
986	GUGUUU A UUGGCA
988	GUUUUU U AGGCAU
989	UAGUUU A GGCAU
1007	CUUUUU A GGCAU
1013	UAGGCAU A AUGGGAG
1024	GGAGAGU A CAGAGGU
1032	CAGAGGU A CACCGAG
1044	GAGGAAU C AAGAUU
1050	UCAAGAU C UAUUUA
1052	AAGAUU A UAUUUA
1054	GAUUUU A UAUUUA
1072	AAGGCAU A UGUGUA
1085	AACAACU C AAAGAAA
1103	GUGUUU U AACUACA
1104	UGUGUU A ACUACAG
1108	AUUUUU A CAGUGUA
1115	ACAGUGU A CUAGACU
1118	GUGUUU A GACUUA
1123	CUAGACU U GACAGCA
1139	AAGAAU A GAGGCUA
1146	AGAGGCU A UCAACA
1148	AGGCUU C AAACAU
1155	CAACAU C AGCUUA
1160	AUCAGCU U AUUCAA
1161	UCAGCUU A AUUCAA
1164	GUUUUU C CAAAGA
1173	AAAGAU A AUGAUU
1181	AUGAUU A GAGCUU
1187	UAGAGCU U UGAGUA
1188	AGAGCUU U GAGUUA
1193	UUUGAGU U AAUAAA
1194	UUGAGU A AAUAAA

Table 36: RSV (N) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
9	AUCUUG CUGAUGAGGCOGAAAGGCOGAA AUUUGCC
21	UUGCUAA CUGAUGAGGCOGAAAGGCOGAA AGCCAUU
23	CUUUGCU CUGAUGAGGCOGAAAGGCOGAA AGAGCCA
24	ACUUGCC CUGAUGAGGCOGAAAGGCOGAA AAGAGCC
32	UCAACUU CUGAUGAGGCOGAAAGGCOGAA ACUUGCC
37	AUCAUUC CUGAUGAGGCOGAAAGGCOGAA ACUUGAC
45	UUGAGUG CUGAUGAGGCOGAAAGGCOGAA AUCAUUC
50	CUUUGUU CUGAUGAGGCOGAAAGGCOGAA AGUGUAU
60	AGAAGUU CUGAUGAGGCOGAAAGGCOGAA AUUUUG
65	AUGACAG CUGAUGAGGCOGAAAGGCOGAA AGUUGAU
66	GAUGACA CUGAUGAGGCOGAAAGGCOGAA AAGUUGA
70	GCOGGAU CUGAUGAGGCOGAAAGGCOGAA ACAGAAG
73	UUUGCUG CUGAUGAGGCOGAAAGGCOGAA AUGACAG
82	GAUGGUG CUGAUGAGGCOGAAAGGCOGAA AUUUGCU
89	UCCGUUG CUGAUGAGGCOGAAAGGCOGAA AUGGUGU
108	UCAAUAC CUGAUGAGGCOGAAAGGCOGAA AUUUUU
111	GUAUCAA CUGAUGAGGCOGAAAGGCOGAA ACUADCU
113	GAGUADC CUGAUGAGGCOGAAAGGCOGAA AUACUUA
117	UAAGGAG CUGAUGAGGCOGAAAGGCOGAA AUCAUAU
120	UAUUUAG CUGAUGAGGCOGAAAGGCOGAA AGUAUCA
123	UCAUAUU CUGAUGAGGCOGAAAGGCOGAA AGGAGUA
126	ACAUCAU CUGAUGAGGCOGAAAGGCOGAA AUUAGGA
127	CACUCCA CUGAUGAGGCOGAAAGGCOGAA AAUUAGG
146	ACUUAUU CUGAUGAGGCOGAAAGGCOGAA AUGUGUU
150	CAUAAU CUGAUGAGGCOGAAAGGCOGAA AUUGAUG
154	GCCACAU CUGAUGAGGCOGAAAGGCOGAA ACUUAUU
155	UGCCACA CUGAUGAGGCOGAAAGGCOGAA AACUUAU
166	GAUUUAU CUGAUGAGGCOGAAAGGCOGAA ACAUGCC
167	UGAUUAA CUGAUGAGGCOGAAAGGCOGAA AACAUUC
169	UGUGAUU CUGAUGAGGCOGAAAGGCOGAA AUUACAU
170	CUUGGAU CUGAUGAGGCOGAAAGGCOGAA AAUAACA
173	CUUCUGU CUGAUGAGGCOGAAAGGCOGAA AUUAUAU
186	UUUUGAU CUGAUGAGGCOGAAAGGCOGAA AGCAUCU
189	AAUUUAU CUGAUGAGGCOGAAAGGCOGAA AUUAGCA
192	GUGAAUU CUGAUGAGGCOGAAAGGCOGAA AUGAUUA
196	CCAGUG CUGAUGAGGCOGAAAGGCOGAA AUUUADG
197	ACCCAGU CUGAUGAGGCOGAAAGGCOGAA AAUUUAU
205	ACCUUAU CUGAUGAGGCOGAAAGGCOGAA ACCCAGU
206	UACCUAU CUGAUGAGGCOGAAAGGCOGAA AACCCAG
209	ACAUAAC CUGAUGAGGCOGAAAGGCOGAA AUUAACC
213	UAUAACA CUGAUGAGGCOGAAAGGCOGAA ACCUAUU

217	OGCAUAT	CUGAUGAGGCOGAAAGGCOGAA	ACAUACC
218	UOGCAUA	CUGAUGAGGCOGAAAGGCOGAA	AACAUAC
220	CADOGCA	CUGAUGAGGCOGAAAGGCOGAA	AUAACAU
229	UAACCUA	CUGAUGAGGCOGAAAGGCOGAA	ACADCGC
231	CCUAACC	CUGAUGAGGCOGAAAGGCOGAA	AGACAUC
235	UCUUCU	CUGAUGAGGCOGAAAGGCOGAA	ACCUAGA
236	CUCUCC	CUGAUGAGGCOGAAAGGCOGAA	AACCUAG
254	GUUUUU	CUGAUGAGGCOGAAAGGCOGAA	AUGGUGU
260	CUCUGAG	CUGAUGAGGCOGAAAGGCOGAA	AUUUUUA
263	CACUCU	CUGAUGAGGCOGAAAGGCOGAA	AGUAUUU
277	UACADGA	CUGAUGAGGCOGAAAGGCOGAA	ADCCCGC
279	UUACAU	CUGAUGAGGCOGAAAGGCOGAA	AUADECC
284	UUGCUUU	CUGAUGAGGCOGAAAGGCOGAA	ACADGAU
299	UUACAU	CUGAUGAGGCOGAAAGGCOGAA	ACUCCAU
305	GUGUGU	CUGAUGAGGCOGAAAGGCOGAA	ACAUUA
315	UCUUGAC	CUGAUGAGGCOGAAAGGCOGAA	AUGUGUU
318	AUGUCU	CUGAUGAGGCOGAAAGGCOGAA	ACGADGU
326	UUCCAU	CUGAUGAGGCOGAAAGGCOGAA	ADGUCUU
327	UUCCAU	CUGAUGAGGCOGAAAGGCOGAA	AADGUCU
346	CACUCA	CUGAUGAGGCOGAAAGGCOGAA	AUUUCAU
347	ACACUUC	CUGAUGAGGCOGAAAGGCOGAA	AADUUA
355	CAUGUU	CUGAUGAGGCOGAAAGGCOGAA	ACACUUC
356	CCAUGU	CUGAUGAGGCOGAAAGGCOGAA	AACACUU
361	GCUUGCC	CUGAUGAGGCOGAAAGGCOGAA	AUGUUAA
370	AGUUGUU	CUGAUGAGGCOGAAAGGCOGAA	AGCUUGC
371	CAGUUGU	CUGAUGAGGCOGAAAGGCOGAA	AAGCUUG
383	UGAUUG	CUGAUGAGGCOGAAAGGCOGAA	AUUUCAG
384	UUGAUUU	CUGAUGAGGCOGAAAGGCOGAA	AADUUA
389	CAUGUU	CUGAUGAGGCOGAAAGGCOGAA	AUUUGAA
395	CUAUCUC	CUGAUGAGGCOGAAAGGCOGAA	ADGUUGA
401	UAGAUUC	CUGAUGAGGCOGAAAGGCOGAA	ADUCUA
406	UUUCUA	CUGAUGAGGCOGAAAGGCOGAA	AUUUAU
408	GADUUC	CUGAUGAGGCOGAAAGGCOGAA	AGAUUCU
415	UUUGUAG	CUGAUGAGGCOGAAAGGCOGAA	AUUUUUU
418	UUUUUG	CUGAUGAGGCOGAAAGGCOGAA	AGGAUUU
431	UUUUUU	CUGAUGAGGCOGAAAGGCOGAA	AGCAUUU
449	CUUGAGC	CUGAUGAGGCOGAAAGGCOGAA	ACCUUCU
453	UAUUCUG	CUGAUGAGGCOGAAAGGCOGAA	AGCUUAC
460	AUGCUG	CUGAUGAGGCOGAAAGGCOGAA	AUUUGG
472	AUCAGGA	CUGAUGAGGCOGAAAGGCOGAA	AGUCAUG
474	CAADCAG	CUGAUGAGGCOGAAAGGCOGAA	AGAGUCA
480	AUCCAC	CUGAUGAGGCOGAAAGGCOGAA	AUCAGGA
491	AUAUAU	CUGAUGAGGCOGAAAGGCOGAA	ADCAUCC
494	UACAUAA	CUGAUGAGGCOGAAAGGCOGAA	AUAUUA
496	UAUACAU	CUGAUGAGGCOGAAAGGCOGAA	AUAUUUU
497	CUAUACA	CUGAUGAGGCOGAAAGGCOGAA	AUAUUUA
501	GUUGCUA	CUGAUGAGGCOGAAAGGCOGAA	ACAUUAU
503	AUGCUG	CUGAUGAGGCOGAAAGGCOGAA	AUAUAUA
511	UAUUAU	CUGAUGAGGCOGAAAGGCOGAA	AUGCUGC

512	UUADUAC	CUGADGAGGCGGAAAGGCGGAA	AADGCTG
515	UAGGUAT	CUGADGAGGCGGAAAGGCGGAA	ACTAATG
518	AUUUAGU	CUGADGAGGCGGAAAGGCGGAA	AUUACTA
522	GCUAAU	CUGADGAGGCGGAAAGGCGGAA	AGUUAU
526	UGGUGU	CUGADGAGGCGGAAAGGCGGAA	AUUUAGU
527	CUGGUG	CUGADGAGGCGGAAAGGCGGAA	AADUAG
544	AAGACCA	CUGADGAGGCGGAAAGGCGGAA	AUCUGUC
549	GUGUAA	CUGADGAGGCGGAAAGGCGGAA	ACCAGAU
551	CGGUGU	CUGADGAGGCGGAAAGGCGGAA	AGACCA
552	ACGGUG	CUGADGAGGCGGAAAGGCGGAA	AAGACCA
563	CUCUGU	CUGADGAGGCGGAAAGGCGGAA	AUCACCG
564	GUGUCC	CUGADGAGGCGGAAAGGCGGAA	AADACCG
573	ACAUUAT	CUGADGAGGCGGAAAGGCGGAA	AGCUCUC
576	AGGACAU	CUGADGAGGCGGAAAGGCGGAA	AUUAGCU
581	UUUUUAG	CUGADGAGGCGGAAAGGCGGAA	ACAUUAT
584	CAUUUU	CUGADGAGGCGGAAAGGCGGAA	AGGACAU
603	CCUUGU	CUGADGAGGCGGAAAGGCGGAA	ACGUUUC
604	GCCUUG	CUGADGAGGCGGAAAGGCGGAA	AACGUUU
613	GGGUAG	CUGADGAGGCGGAAAGGCGGAA	AGGUUUU
614	UGGUAG	CUGADGAGGCGGAAAGGCGGAA	AAGGUUU
617	CCUUGG	CUGADGAGGCGGAAAGGCGGAA	AGUAGG
629	UGUUGG	CUGADGAGGCGGAAAGGCGGAA	AUGUCCU
640	UUCAUAG	CUGADGAGGCGGAAAGGCGGAA	AGGUUUU
641	CUUCAU	CUGADGAGGCGGAAAGGCGGAA	AAGGUUU
643	CACUCA	CUGADGAGGCGGAAAGGCGGAA	AGAAGCU
652	UUUUCA	CUGADGAGGCGGAAAGGCGGAA	ACACUUC
653	GUUUUC	CUGADGAGGCGGAAAGGCGGAA	AACACUU
663	AAGUGG	CUGADGAGGCGGAAAGGCGGAA	AUGUUUU
670	AUCUUA	CUGADGAGGCGGAAAGGCGGAA	AGUGGG
671	CAUCUA	CUGADGAGGCGGAAAGGCGGAA	AAGUGG
672	ACAUUA	CUGADGAGGCGGAAAGGCGGAA	AAGUGG
674	AAACAU	CUGADGAGGCGGAAAGGCGGAA	AUAAAGU
680	GAACAA	CUGADGAGGCGGAAAGGCGGAA	ACAUUA
681	UGAACAA	CUGADGAGGCGGAAAGGCGGAA	AACAUU
682	AUGAAC	CUGADGAGGCGGAAAGGCGGAA	AAACAU
683	AADGAC	CUGADGAGGCGGAAAGGCGGAA	AAACAU
686	CAAAAU	CUGADGAGGCGGAAAGGCGGAA	ACAAAA
687	OCAAAA	CUGADGAGGCGGAAAGGCGGAA	ACAAAA
690	AUACCA	CUGADGAGGCGGAAAGGCGGAA	AUGAAC
691	UAUACA	CUGADGAGGCGGAAAGGCGGAA	AADGAC
692	CUAUAC	CUGADGAGGCGGAAAGGCGGAA	AAUGAA
696	UGUGUA	CUGADGAGGCGGAAAGGCGGAA	ACAAAA
698	AUGUGG	CUGADGAGGCGGAAAGGCGGAA	AUACCA
706	GGUAGA	CUGADGAGGCGGAAAGGCGGAA	AUGUGG
708	CUGGUA	CUGADGAGGCGGAAAGGCGGAA	AGAUUGU
709	UCUGUA	CUGADGAGGCGGAAAGGCGGAA	AAGAUUG
711	CCUUGG	CUGADGAGGCGGAAAGGCGGAA	AGAAGAU
726	UCAACU	CUGADGAGGCGGAAAGGCGGAA	ACUGCA
731	UCCUUC	CUGADGAGGCGGAAAGGCGGAA	ACUCUAC

740	CUGCAA	CUGAUGAGGCOGAAAGGCOGAA	AUCCCUU
741	CCUGCA	CUGAUGAGGCOGAAAGGCOGAA	AAUCCCU
742	UCCUGCA	CUGAUGAGGCOGAAAGGCOGAA	AAAUCCC
743	AUCCUGC	CUGAUGAGGCOGAAAGGCOGAA	AAAADCC
751	CAUAAAC	CUGAUGAGGCOGAAAGGCOGAA	AUCCUGC
754	AUUCAU	CUGAUGAGGCOGAAAGGCOGAA	ACAADCC
755	CAUUCAU	CUGAUGAGGCOGAAAGGCOGAA	AACAADC
756	GCACCA	CUGAUGAGGCOGAAAGGCOGAA	AAACAAU
766	UGCACA	CUGAUGAGGCOGAAAGGCOGAA	AGGCADU
787	CCACCG	CUGAUGAGGCOGAAAGGCOGAA	ACAUCC
788	CCACCG	CUGAUGAGGCOGAAAGGCOGAA	AACADCA
800	UUGCUA	CUGAUGAGGCOGAAAGGCOGAA	ACUCCCC
802	UUUGCU	CUGAUGAGGCOGAAAGGCOGAA	AGACUCC
803	AUUUGC	CUGAUGAGGCOGAAAGGCOGAA	AAGACUC
811	UUUAACU	CUGAUGAGGCOGAAAGGCOGAA	AUUUUGC
815	UAUUUU	CUGAUGAGGCOGAAAGGCOGAA	ACUGADU
816	AUAUUU	CUGAUGAGGCOGAAAGGCOGAA	AACUGAU
822	AACAUA	CUGAUGAGGCOGAAAGGCOGAA	AUUUUUA
824	CUAACAU	CUGAUGAGGCOGAAAGGCOGAA	AUAUUUU
825	CCUACA	CUGAUGAGGCOGAAAGGCOGAA	AUAUUUU
829	AUGUCCU	CUGAUGAGGCOGAAAGGCOGAA	ACAUAAU
830	CAUGUC	CUGAUGAGGCOGAAAGGCOGAA	AACAUA
840	UGCACAC	CUGAUGAGGCOGAAAGGCOGAA	AGCAUGU
866	CCUACAC	CUGAUGAGGCOGAAAGGCOGAA	ACUUGUU
869	AAACUC	CUGAUGAGGCOGAAAGGCOGAA	ACAACUU
875	AUUCUA	CUGAUGAGGCOGAAAGGCOGAA	ACCUCAA
876	UAUUCAU	CUGAUGAGGCOGAAAGGCOGAA	AACUCA
877	AUAUUA	CUGAUGAGGCOGAAAGGCOGAA	AAACUCC
883	UUGGCA	CUGAUGAGGCOGAAAGGCOGAA	AUUCUA
895	ACCACCC	CUGAUGAGGCOGAAAGGCOGAA	AUUUUUG
913	AUGGUAG	CUGAUGAGGCOGAAAGGCOGAA	AUCCUGC
914	UAUGUA	CUGAUGAGGCOGAAAGGCOGAA	AUCCUG
916	UAUAUG	CUGAUGAGGCOGAAAGGCOGAA	AGAUCC
921	UUAUA	CUGAUGAGGCOGAAAGGCOGAA	AUGGUAG
923	UGUUA	CUGAUGAGGCOGAAAGGCOGAA	AUAUGGU
925	GUUGUUC	CUGAUGAGGCOGAAAGGCOGAA	AUAUAUG
943	UAUAUAU	CUGAUGAGGCOGAAAGGCOGAA	AUGUUUU
946	AGAUAAU	CUGAUGAGGCOGAAAGGCOGAA	AUGAUGC
947	AAGUA	CUGAUGAGGCOGAAAGGCOGAA	AUGAUG
949	CAAAGAU	CUGAUGAGGCOGAAAGGCOGAA	AUAADGA
950	UCAAGA	CUGAUGAGGCOGAAAGGCOGAA	AUAADUG
952	AGUCAA	CUGAUGAGGCOGAAAGGCOGAA	AUAADUA
954	UGAGUA	CUGAUGAGGCOGAAAGGCOGAA	AGAUAAU
955	UGAGUC	CUGAUGAGGCOGAAAGGCOGAA	AAGAUAA
960	GAUAUU	CUGAUGAGGCOGAAAGGCOGAA	AGUCAA
964	GUGAGGA	CUGAUGAGGCOGAAAGGCOGAA	AUGAGU
965	AGUGAGG	CUGAUGAGGCOGAAAGGCOGAA	AUUGAG
966	AAGUGAG	CUGAUGAGGCOGAAAGGCOGAA	AAAUUGA
969	GAGAAGU	CUGAUGAGGCOGAAAGGCOGAA	AGGAUU

973	ACUGGAG	CUGAUGAGGCGGAAAGGCGGAA	AGUGAGG
974	CACUGGA	CUGAUGAGGCGGAAAGGCGGAA	AAGUGAG
976	UACACUG	CUGAUGAGGCGGAAAGGCGGAA	AGAAGUG
983	CUAUAC	CUGAUGAGGCGGAAAGGCGGAA	ACACUGG
986	UGOCUAA	CUGAUGAGGCGGAAAGGCGGAA	ACTACAC
988	AUUGOCU	CUGAUGAGGCGGAAAGGCGGAA	ATACTAC
989	CAUUGCC	CUGAUGAGGCGGAAAGGCGGAA	AATACTA
1007	UUAUGCC	CUGAUGAGGCGGAAAGGCGGAA	AGGOCAG
1013	CUCCCAU	CUGAUGAGGCGGAAAGGCGGAA	ADGCCUA
1024	AOCUCUG	CUGAUGAGGCGGAAAGGCGGAA	ACUCGCC
1032	CUCCGUG	CUGAUGAGGCGGAAAGGCGGAA	ACCCUGG
1044	AGAUUUU	CUGAUGAGGCGGAAAGGCGGAA	AUUCUUC
1050	UCAUUA	CUGAUGAGGCGGAAAGGCGGAA	ADCUUGA
1052	CAUCAUA	CUGAUGAGGCGGAAAGGCGGAA	AGAUCUU
1054	UGCAUCA	CUGAUGAGGCGGAAAGGCGGAA	ADAGADC
1072	UUCAGCA	CUGAUGAGGCGGAAAGGCGGAA	ADGCCUU
1085	UUUCUUU	CUGAUGAGGCGGAAAGGCGGAA	AGUUGUU
1103	UGUAGUU	CUGAUGAGGCGGAAAGGCGGAA	AUCACAC
1104	CUGUAGU	CUGAUGAGGCGGAAAGGCGGAA	AADCAUA
1108	UACACUG	CUGAUGAGGCGGAAAGGCGGAA	AGUUAUU
1115	AGOCUAG	CUGAUGAGGCGGAAAGGCGGAA	ACACUGU
1118	UCAAGUC	CUGAUGAGGCGGAAAGGCGGAA	AGUACAC
1123	UGCUGUC	CUGAUGAGGCGGAAAGGCGGAA	AGCCUAG
1139	UAGCCUC	CUGAUGAGGCGGAAAGGCGGAA	AGUUCUU
1146	UGUUUGA	CUGAUGAGGCGGAAAGGCGGAA	AGCCUCU
1148	GADGUUU	CUGAUGAGGCGGAAAGGCGGAA	AUAGCCU
1155	UUAAGCU	CUGAUGAGGCGGAAAGGCGGAA	AUGUUUG
1160	UUGGAUU	CUGAUGAGGCGGAAAGGCGGAA	AGCUGAU
1161	UUUGGAU	CUGAUGAGGCGGAAAGGCGGAA	AAGCUGA
1164	UCUUUUG	CUGAUGAGGCGGAAAGGCGGAA	AUUAAGC
1173	ACAUCAU	CUGAUGAGGCGGAAAGGCGGAA	AUCUUUU
1181	AAAGCUC	CUGAUGAGGCGGAAAGGCGGAA	ACAUCAU
1187	UAACUCA	CUGAUGAGGCGGAAAGGCGGAA	AGCUCUA
1188	UUAACUC	CUGAUGAGGCGGAAAGGCGGAA	AAGCUCU
1193	UUUUUAU	CUGAUGAGGCGGAAAGGCGGAA	ACUCAAA
1194	UUUUUAU	CUGAUGAGGCGGAAAGGCGGAA	AACUCAU

Table 87: RSV (1B) HP Ribozyme/Substrate Sequence

nt. Position	HP Ribozyme Sequence	Substrate
70	CUGUGAUC AGAA GUCUUU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AAAGACU GAU GAUCACAG
91	CAAGUGAC AGAA GUCUUA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UGAGACC GUU GUCACUUG
472	CAGGCUCC AGAA GAGCUA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UAGUCCA GAU GAGGCUUG

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Table 88: RSV (N) HP Ribozyme/Substrate Sequence

nt. Position	HP Ribozyme Sequence	Substrate
476	NUCCACAC AGAA GAGAGG ACCAGAGAGACACACGUGUGUGACAUUACCUAGUA	CUCUCCU GAU UGUGGGAU
540	AGGACCAAG AGAA GUCCCC ACCAGAGAGACACACGUGUGUGACAUUACCUAGUA	GGGAGCA GAU CUGGUCUU
554	CUAAGUCAC AGAA GUAGGA ACCAGAGAGACACACGUGUGUGACAUUACCUAGUA	UCUUACA GCC GUGAUAUAG
636	UUCAUAGA AGAA GUUGGC ACCAGAGAGACACACGUGUGUGACAUUACCUAGUA	GGCAACA GCU UCUAUAUA
998	CCUAGGCC AGAA GCUUUG ACCAGAGAGACACACGUGUGUGACAUUACCUAGUA	CAUUGCU GCU GGGCUAGG
1156	UUGGUAUA AGAA GAUGUU ACCAGAGAGACACACGUGUGUGACAUUACCUAGUA	AAUAUA GCU UUAUCCUA

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Table 39: Large-Scale Synthesis

Sequence	Activator [Added/Final] (min)	Amidite [Added/Final] (min)	Time*	% Full Length Product
A ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	85
A ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	89
(GGU) ₃ GGT	T [0.50/0.33]	[0.1/0.02]	15 m	78
(GGU) ₃ GGT	S [0.25/0.17]	[0.1/0.02]	15 m	81
C ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	90
C ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	97
U ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	80
U ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	85
A (36-mer)	T [0.50/0.33]	[0.1/0.02]	15/15m	21
A (36-mer)	S [0.25/0.17]	[0.1/0.02]	15/15 m	25
A (36-mer)	S [0.50/0.24]	[0.1/0.03]	15/15 m	25
A (36-mer)	S [0.50/0.18]	[0.1/0.05]	15/15 m	38
A (36-mer)	S [0.50/0.18]	[0.1/0.05]	10/5 m	42

*Where two coupling times are indicated the first refers to RNA coupling and the second to 2'-O-methyl coupling. S = 5-S-Ethyltetrazole, T = tetrazole activator. A is 5' -ucU ccA UCU GAU GAG GCC GAA AGG CCG AAA Auc ccu -3' where lowercase represents 2'-O-methylnucleotides.

Table 40: Base Deprotection

Sequence	Deprotection Reagent	Time (min)	T °C	% Full Length Product
iBu(GGU) ₄	NH ₄ OH/EtOH	16 h	55	62.5
	MA	10 m	65	62.7
	AMA	10 m	65	74.8
	MA	10 m	55	75.0
	AMA	10 m	55	77.2
iPrP(GGU) ₄	NH ₄ OH/EtOH	4 h	65	44.8
	MA	10 m	65	65.9
	AMA	10 m	65	59.8
	MA	10 m	55	61.3
	AMA	10 m	55	60.1
C ₉ U	NH ₄ OH/EtOH	4 h	65	75.2
	MA	10 m	65	79.1
	AMA	10 m	65	77.1
	MA	10 m	55	79.8
	AMA	10 m	55	75.5
A (36-mer)	NH ₄ OH/EtOH	4 h	65	22.7
	MA	10 m	65	28.9

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Table 41: 2'-O-Alkylsilyl Deprotection

Sequence	Deprotection Reagent	Time (min)	T °C	% Full Length Product
AgT	TBAF	24 h	20	84.5
	1.4 M HF	0.5 h	65	81.0
(GGU) ₄	TBAF	24 h	20	60.9
	1.4 M HF	0.5 h	65	67.8
C ₁₀	TBAF	24 h	20	86.2
	1.4 M HF	0.5 h	65	86.1
U ₁₀	TBAF	24 h	20	84.8
	1.4 M HF	0.5 h	65	84.5
B (36-mer)	TBAF	24 h	20	25.2
	1.4 M HF	1.5 h	65	30.6
A (36-mer)	TBAF	24 h	20	29.7
	1.4 M HF	1.5 h	65	30.4

B is 5'- UCU CCA UCU GAU GAG GCC GAA AGG CCG AAA AUC CCU
-3'.

Table 42: NMR Data for UC Dimers containing
Phosphorothioate Linkage

Synthesis #	Type	Delivery	Eq.	Wait	ASE (%)
3524	ribo	2 x 3 s	10.4	2 x 100 s	95.9
3525	ribo	2 x 3 s	10.4	2 x 75 s	92.6
3530	ribo	2 x 3 s	10.4	2 x 75 s	92.1
3526	ribo	1 x 5 s	08.6	1 x 300 s	100.0
3578	ribo	1 x 5 s	08.6	1 x 250 s	100.0
3529	ribo	1 x 5 s	08.6	1 x 150 s	73.7

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Table 43: NMR Data for 15-mer RNA containing
Phosphorothioate Linkages

Synthesis #	Type	Delivery	Eq.	Wait	ASE (%)
3581	ribo	1 x 5 s	08.6	1 x 250 s	99.6
3663	ribo	2 x 4 s	13.8	2 x 300 s	100.0
3582	2'-O-Me	1 x 5 s	08.6	1 x 250 s	99.7
3668	2'-O-Me	2 x 4 s	13.8	2 x 300 s	99.8
3682	2'-O-Me	1 x 5 s	08.6	1 x 300 s	99.8

Table 44. Kinetics of Self-Processing *In Vitro*

Self-Processing Constructs	k (min ⁻¹)*
HH	1.16 ± 0.08
HDV	0.56 ± 0.15
HP(GC)	0.36 ± 0.06
HP(GU)	0.054 ± 0.003

* k represents the unimolecular rate constant for ribozyme self-cleavage determined from a non-linear, least-squares fit (KaleidaGraph, Synergy Software, Reading, PA) to the equation:

$$(\text{Fraction Uncleaved Transcript}) = \frac{1}{kt} (1 - e^{-kt})$$

The equation describes the extent of ribozyme processing in the presense of ongoing transcription (Long & Uhlenbeck, 1994 Proc. Natl. Acad. Sci. USA 91, 6977) as a function of time (t) and the unimolecular rate constant for cleavage (k). Each value of k represents the average (± range) of values determined from two experiments.

Table 45

Entry	Modification	$t_{1/2}$ (m) Activity (t_A)	$t_{1/2}$ (m) Stability (t_S)	$\beta = t_S/t_A$ $\times 10$
1	U4 & U7 = U	1	0.1	1
2	U4 & U7 = 2'-O-Me-U	4	260	650
3	U4 = 2'-CH ₂ -U	6.5	120	180
4	U7 = 2'-CH ₂ -U	8	280	350
5	U4 & U7 = 2'-CH ₂ -U	9.5	120	130
6	U4 = 2'-CF ₂ -U	5	320	640
7	U7 = 2'-CF ₂ -U	4	220	550
8	U4 & U7 = 2'-CF ₂ -U	20	320	160
9	U4 = 2'-F-U	4	320	800
10	U7 = 2'-F-U	8	400	500
11	U4 & U7 = 2'-F-U	4	300	750
12	U4 = 2'-C-Allyl-U	3	>500	>1700
13	U7 = 2'-C-Allyl-U	3	220	730
14	U4 & U7 = 2'-C-Allyl-U	3	120	400
15	U4 = 2'-araF-U	5	>500	>1000
16	U7 = 2'-araF-U	4	350	875
17	U4 & U7 = 2'-araF-U	15	500	330
18	U4 = 2'-NH ₂ -U	10	500	500
19	U7 = 2'-NH ₂ -U	5	500	1000
20	U4 & U7 = 2'-NH ₂ -U	2	300	1500
21	U4 = dU	6	100	170
22	U4 & U7 = dU	4	240	600

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CLAIMSWhat is claimed is:

1. An enzymatic nucleic acid molecule which cleaves ICAM-1 mRNA, IL-5 mRNA, *rel A* mRNA, TNF- α mRNA sites shown in Table 23, 25, 27, or 28, CML associated mRNA selected from those identified as SEQ. ID NOS 1-25, or RSV mRNA or RSV genomic RNA in a region selected from the group consisting of 1C, 1B and N.
2. The enzymatic nucleic acid molecule of claim 1, the binding arms of which contain sequences complementary to any one of the sequences defined in any of those in Tables 2, 3, 6-9, 11, 13, 15-23, 27, 28, 31, 33, 34, 36, and 37.
3. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said nucleic acid molecule is in a hammerhead motif.
4. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said RNA molecule is in a hairpin, hepatitis delta virus, group 1 intron, *Neurospora* VS RNA or RNaseP RNA motif.
5. The enzymatic nucleic acid molecule of claim 1 or 2, comprising between 12 and 100 bases complementary to said mRNA or genomic RNA.
6. The enzymatic nucleic acid molecule of claim 5 comprising between 14 and 24 bases complementary to said mRNA or genomic RNA.
7. The enzymatic nucleic acid molecule of claim 1 or 2, comprising between 5 and 23 bases complementary to said mRNA or genomic RNA.
8. The enzymatic nucleic acid molecule of claim 7 comprising between 10 and 18 bases complementary to said mRNA or genomic RNA.
9. An enzymatic nucleic acid molecule consisting essentially of a sequence selected from the group of those shown in Tables 4-8, 10, 12, 14-16, 19-22, 24, 26-28, 30, 32, 34 and 36-38.
10. A mammalian cell including an enzymatic nucleic acid molecule of claims 1 or 2.

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11. The cell of claim 10, wherein said cell is a human cell.
12. An expression vector including nucleic acid encoding an enzymatic nucleic acid molecule or multiple enzymatic molecules of claims 1 or 2 in a manner which allows expression of that enzymatic RNA molecule(s) within a mammalian cell.
13. A mammalian cell including an expression vector of claim 12.
14. The cell of claim 13, wherein said cell is a human cell.
15. A method for treatment of a pathological condition related to the mRNA level of ICAM-1, IL-5, *rel A*, TNF- α , or RSV by administering to a patient an enzymatic nucleic acid molecule of claim 1 or 2.
16. A method for treatment of a pathological condition related to the mRNA level of ICAM-1, IL-5, *rel A*, TNF- α , or RSV by administering to a patient an expression vector of claim 12.
17. The method of claims 15 or 16, wherein said patient is a human.
18. The method of claim 17 wherein said condition is selected from the group consisting of atherosclerosis, myocardial infraction, stroke, restenosis, heart diseases, cancer, rheumatoid arthritis, asthma, reperfusion injury, inflammatory or autoimmune disorders, transplant rejection, myocardial ischemia, stroke, psoriasis, Kawasaki disease, HIV and AIDS, and septic shock.
19. A nucleoside selected from the group consisting of 5'-C-alkylnucleoside, 2'-deoxy-2'-alkylnucleoside, nucleoside 5'-deoxy-5'-dihalo-methylphosphonate, nucleoside 5'-deoxy-5'-difluoro-methylphosphonate, nucleoside 3'-deoxy-3'-dihalo-methylphosphonate, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
20. A nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.

21. A nucleotide triphosphate comprising a nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
22. The 5'-C-alkylnucleoside of claim 19, wherein the sugar portion is in a talo configuration.
23. The 5'-C-alkylnucleoside of claim 19, wherein the sugar portion is in an allo configuration.
24. An oligonucleotide comprising a nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
25. An oligonucleotide comprising a moiety having the formula:
- wherein B is a nucleotide base or hydrogen; R1, R2 and R3 independently is selected from the group consisting of hydrogen, an alkyl group containing between 2 and 10 carbon atoms inclusive, an amine, an amino acid, and a peptide containing between 2 and 5 amino acids inclusive; and the zigzag lines are independently hydrogen or a bond.
26. An oligonucleotide comprising a 3'-amido or peptido group.
27. An oligonucleotide comprising a 5'-amido or peptido group.
28. The oligonucleotide of claim 24, 25, 26, or 27 having enzymatic activity.
29. Method for producing an enzymatic nucleic acid molecule having activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one nucleotide having an alkyl group at its 5'-position or 2'-position.

- 5 30. Method for conversion of a protected allo sugar to a protected talo sugar, comprising the step of contacting said protected allo sugar with triphenyl phosphine, diethylazodicarboxylate, p-nitrobenzoic acid under inversion causing conditions to provide said protected talo sugar.
- 10 31. Method for the synthesis of a nucleoside 5' or a 3'-dihalo-methylphosphonate comprising the step of condensing a difluoromethylphosphonate-containing sugar with a pyrimidine or purine under conditions suitable for forming a nucleoside 5'- or 3'-difluoromethylphosphonate.
- 15 32. The oligonucleotide of claim 3, wherein the normal hammerhead U4 and/or U7 positions are substituted with 2'-NH-amino acid.
33. A method for the synthesis of RNA comprising the step of providing 5-S-alkyltetrazole at a delivered 0.1-1.0 M concentration for the activation of a RNA amidite during a coupling step for less than or equal to 10 minutes.
- 20 34. A method for the synthesis of RNA comprising the step of providing 5-S-alkyltetrazole at 0.15-0.35 M effective, or final, concentration for the activation of a RNA amidite during a coupling step for less than or equal to 10 minutes.
- 25 35. A method for the deprotection of RNA comprising the step of providing alkylamine (MA) or NH₄OH/alkylamine (AMA) at between 60°C - 70°C for 5 to 15 minutes to remove any exocyclic amino protecting groups from protected RNA; wherein said alkyl is selected from the group consisting of methyl, ethyl, propyl and butyl.
- 30 36. A method for the deprotection of RNA alkylsilyl protecting groups comprising, contacting said groups with anhydrous triethylamine•hydrogen fluoride (aHF•TEA) trimethylamine or diisopropylethylamine at between 60 °C-70 °C for 0.25-24 h.
37. A method for the purification of an RNA molecule by passing said enzymatic RNA molecule over an HPLC column, wherein said HPCC column is an anion exchange chromatography column.

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38. Method for one pot deprotection of RNA comprising, contacting a protected base with anhydrous methyl amine at between 60 °C-70 °C for at least 5 min, cooling the resulting mixture and contacting said mixture with TEA-3HF reagents under conditions which remove a protecting group of the 2'-hydroxyl position.
39. Method for synthesizing RNA containing a phosphorothioate linkage comprising the step of contacting 6-10 equivalents of 3H-1,2-benzodithiole-3-one 1,1-dioxide (Beaucage reagent) with the growing RNA chain for 5 seconds with a reaction time of at least 300 seconds.
40. Method of synthesizing RNA containing a phosphorothioate linkage comprising the step of achieving coupling with 5-S-ethyltetrazole or 5-S-methyltetrazole prior to sulfurization.
41. Method of claims 38, 39 or 40 wherein said RNA is enzymatically active.
42. Method for synthesizing 2'-deoxy-2'-amino-nucleoside phosphoramidite, comprising the step of protecting the 2'-amino group with a N-phtaloyl group.
43. The method of claim 42 wherein the said nucleoside lacks a base.
44. Method for synthesis of RNA comprising the step of: protecting the 2'-position of a nucleotide during said synthesis with a (trimethylsilyl)ethoxymethyl (SEM) group.
45. Method for covalently linking a SEM group to the 2'-position of a nucleotide, comprising the step of: contacting a nucleoside with an SEM-containing molecule under SEM bonding conditions.
46. The method of claim 45, wherein said conditions comprise dibutyltin oxide and tetrabutylammonium fluoride and SEM-Cl.
47. Method for removal of an SEM group from a nucleoside molecule or an oligonucleotide, comprising the step of: contacting said molecule or oligonucleotide with boron trifluoride etherate (BF₃•OEt₂) under SEM removing conditions.

48. The method of claim 57 wherein said (BF₃·OEt₂) is provided in acetonitrile.
49. One or more vectors comprising
- 5 a first nucleic acid sequence encoding a first ribozyme having intramolecular or intermolecular cleaving activity, said first ribozyme being selected from the group consisting of a hammerhead, hairpin, hepatitis delta virus, *Neurospora* VS RNA, Group I, and RNaseP motif;
- 10 and a second nucleic acid sequence encoding a second ribozyme having intermolecular cleaving activity, said Second ribozyme being selected from the group consisting of a hammerhead, hairpin, hepatitis delta virus, *Neurospora* VS RNA, Group I, and RNaseP motif and said second nucleic acid being flanked by other
- 15 nucleic acid sequences encoding RNA which is cleaved by said first ribozyme to release said second ribozyme from RNA encoded by said vector;
- 20 wherein said first and second nucleic acid sequences may be on the same or separate nucleic acid molecules, and said vector encodes mRNA or comprises RNA which lacks secondary structure which reduces release of said second ribozyme by more than 20%.
50. Cell comprising the vector of claim 49.
51. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions
- 25 between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs.
52. The RNA molecule of claim 51, wherein said molecule is transcribed by a RNA polymerase III based promoter system.
53. The RNA molecule of claim 51, wherein said molecule is transcribed
- 30 by a type 2 pol III promoter system.
54. The RNA molecule of claim 51, wherein said molecule is a chimeric tRNA.

55. The RNA molecule of claim 53, said RNA having A and B boxes of a type 2 pol III promoter separated by between 0 and 300 bases.
56. The RNA molecule of claim 53, wherein said desired RNA molecule is at the 3' end of said B box.
- 5 57. The RNA molecule of claim 53, wherein said desired RNA molecule is in between the said A and the B box.
58. The RNA molecule of claim 53, wherein said desired RNA molecule includes said B box.
- 10 59. The RNA molecule of claim 51, wherein said desired RNA molecule is selected from the group consisting of antisense RNA, decoy RNA, therapeutic editing RNA, enzymatic RNA, agonist RNA and antagonist RNA.
60. The RNA molecule of claim 51, wherein said 5' terminus is able to base-pair with at least 12 bases of said 3' region.
- 15 61. The RNA molecule of claim 51, wherein said 5' terminus is able to base-pair with at least 15 bases of said 3' region.
62. DNA vector encoding the RNA molecule of claim 51
63. The vector of claim 62, wherein said vector is derived from an AAV or adeno virus.
- 20 64. RNA vector encoding the RNA molecule of claim 51.
65. The vector of claim 64, wherein said vector is derived from an alpha virus or retro virus.
66. The vector of claim 62 wherein the portions of the vector encoding said RNA function as a RNA pol III promoter.
- 25 67. Cell comprising the vector of claim 62.
68. Cell comprising the vector of claim 53.
69. Cell comprising the RNA of claim 51.

70. Method to provide a desired RNA molecule in a cell, comprising introducing said molecule into said cell a RNA comprising a desired RNA molecule, having a 5' terminus able to base pair with at least 8 bases of a 3' region of said RNA molecule.
- 5 71. The method of claim 70, wherein said introducing comprises providing a vector encoding said RNA molecule.
72. Hammerhead ribozyme having 2 or 3 base pairs in stem II with an interconnecting loop of 4 or more bases between said base pairs.
- 10 73. Hairpin ribozyme lacking a substrate moiety, comprising at least six bases in helix 2 and able to base-pair with a separate substrate RNA, wherein the said ribozyme comprises one or more bases 3' of helix 3 able to base-pair with the said substrate RNA to form a helix 5 and wherein the said ribozyme can cleave and/or ligate said separate RNA(s) in *trans*.
- 15 74. The ribozyme of claim 73, wherein said ribozyme comprises six bases in helix 2.
75. The ribozyme of claim 73, having the structure of Fig. 3, wherein each N and N' is independently any base and each dash may represent a hydrogen bond, r is 1-20, q is 2-20, o is 0 - 20, n is 1 - 4, and m is 1 - 20.
- 20 76. Method for increasing the activity of a hairpin ribozyme by providing one or more bases 3' of helix 3 able to base-pair with a substrate RNA to form a helix 5.
- 25 77. Trans-cleaving Hairpin ribozyme comprising at least 6 base pairs in helix 2 lacking a substrate RNA moiety.
78. Trans-ligating Hairpin ribozyme comprising at least 6 base pairs in helix 2 lacking a substrate RNA moiety.
79. The ribozyme of claim 73 having the structure of Fig. 73.
80. The ribozyme of claim 73 having the structure of Fig. 74.
- 30 81. A cell including the ribozyme of any of claims 73-80.

82. An expression vector comprising nucleic acid encoding the ribozyme of any of claims 73-80, in a manner which allows expression of that ribozyme within a cell.
83. A cell including an expression vector of claim 82.
- 5 84. Method for altering in vivo the nucleotide base sequence of a naturally occurring mutant nucleic acid molecule, comprising the steps of:
- 10 contacting said nucleic acid molecule in vivo with an oligonucleotide or peptide nucleic acid able to form a duplex or triplex molecule with said nucleic acid molecule, wherein formation of said duplex or triplex molecule directly, or after nucleic acid repair *in vivo*, causes at least one base in said nucleic acid molecule to be chemically modified to functionally alter the nucleotide base sequence of said nucleic acid sequence.
- 15 85. The method of claim 84, wherein said oligonucleotide is of a length sufficient to activate dsRNA deaminase in vivo to cause conversion of an adenine base to inosine in an RNA molecule.
- 20 86. The method of claim 84, wherein said oligonucleotide comprises an enzymatic nucleic acid molecule which is active to chemically modify a base.
87. The method claim 84, wherein said nucleic acid molecule is DNA or RNA.
88. The method of claim 84, wherein said oligonucleotide comprises a chemical mutagen.
- 25 89. The method of claim 88, wherein said mutagen is nitrous acid.
90. The method of claim 84 wherein said oligonucleotide causes deamination of 5-methylcytosine to thymidine, cytosine to uracil, or adenine to inosine, or methylation of cytosine to 5-methylcytosine.
- 30 91. The method of claim 84, wherein an endogenous mammalian editing system is co-opted to cause said chemical modification.

92. Method for introduction of enzymatic nucleic acid into a cell or tissue, comprising the steps of;

5 providing a complex of a first nucleic acid molecule encoding said enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

10

and contacting said complex with said cell or tissue under conditions in which said enzymatic nucleic acid molecule is produced in said cell or tissue.

- 15 93. Method for introduction of a desired nucleic acid into a cell or tissue, comprising the steps of;

20 providing a complex of a first nucleic acid molecule encoding said desired nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said first nucleic acid molecule lacks a promoter region and said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

25 and contacting said complex with said cell or tissue under conditions in which said desired acid molecule is produced in said cell or tissue.

- 94 Method for introduction of a desired nucleic acid into a cell or tissue, comprising the steps of;

30 providing a complex of a first nucleic acid molecule encoding said enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired

structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

5 and wherein said second nucleic acid further comprises a localization factor;

and contacting said complex with said cell or tissue under conditions in which said desired nucleic acid molecule is produced in said cell or tissue.

10 95. Complex of a first nucleic acid molecule encoding an enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule;
15 wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.

20 96. Complex of a first nucleic acid molecule encoding a desired nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule;
25 wherein said first nucleic acid molecule lacks a promoter region and said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.

30 97. Complex of a first nucleic acid molecule encoding an enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule;
wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said

first nucleic acid under said conditions, and wherein said second nucleic acid further comprises a localization factor.

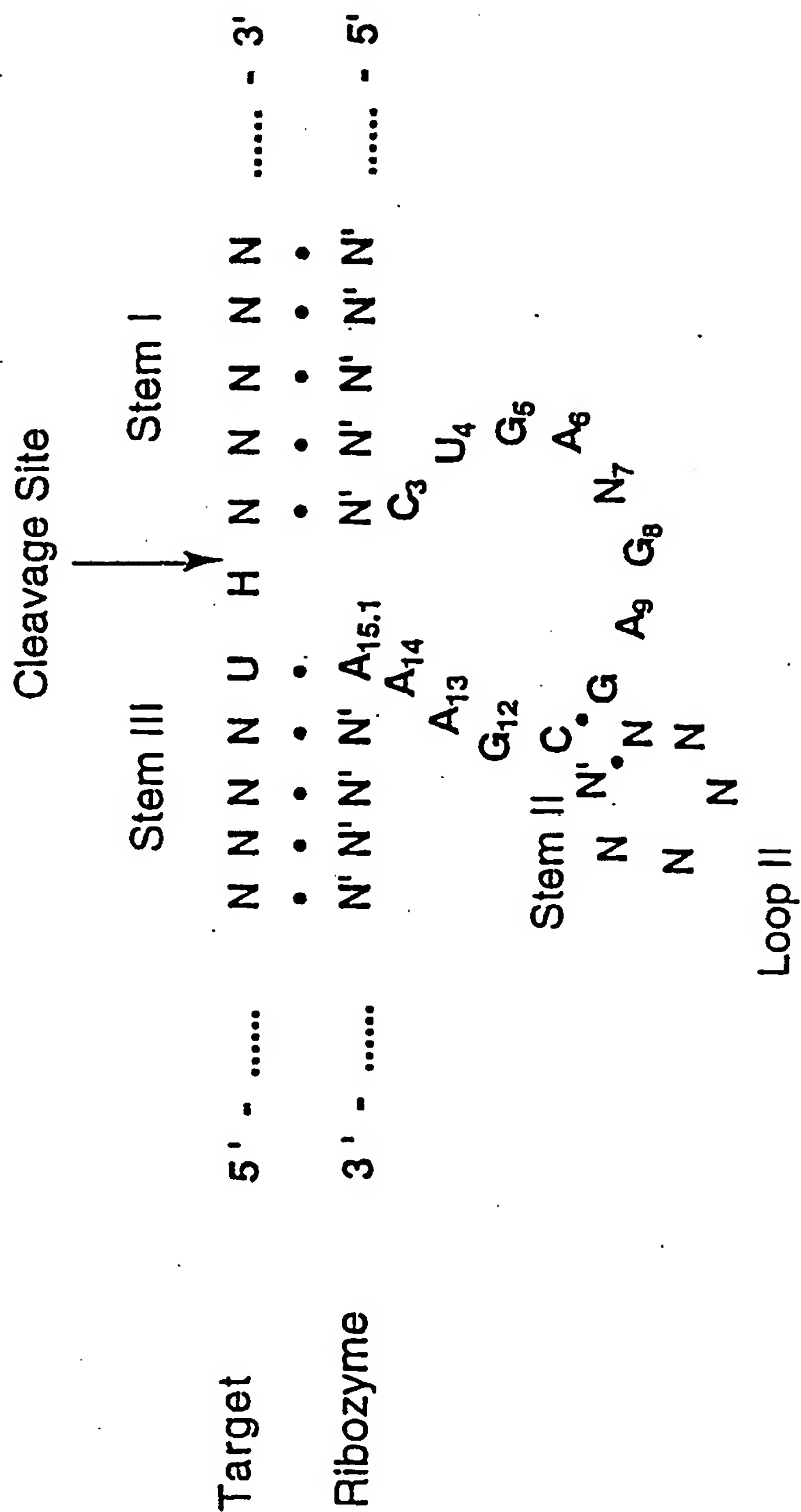
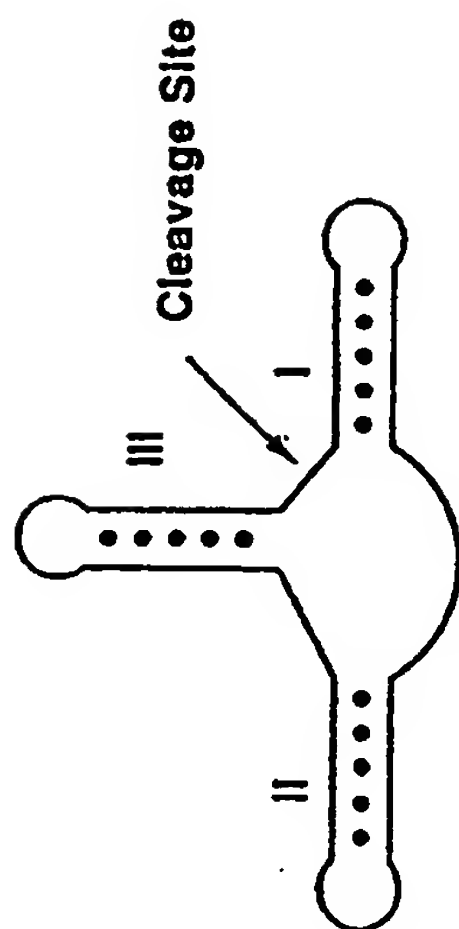


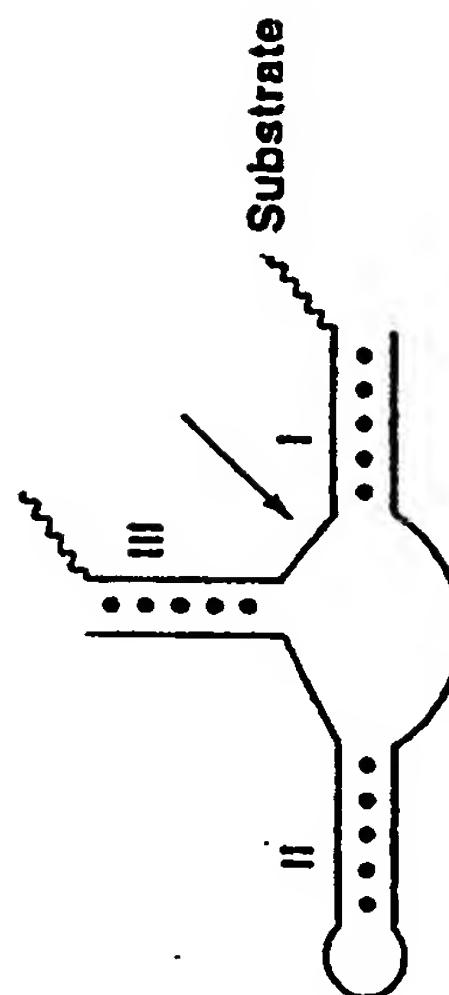
FIG. 1.

FIG. 2a.



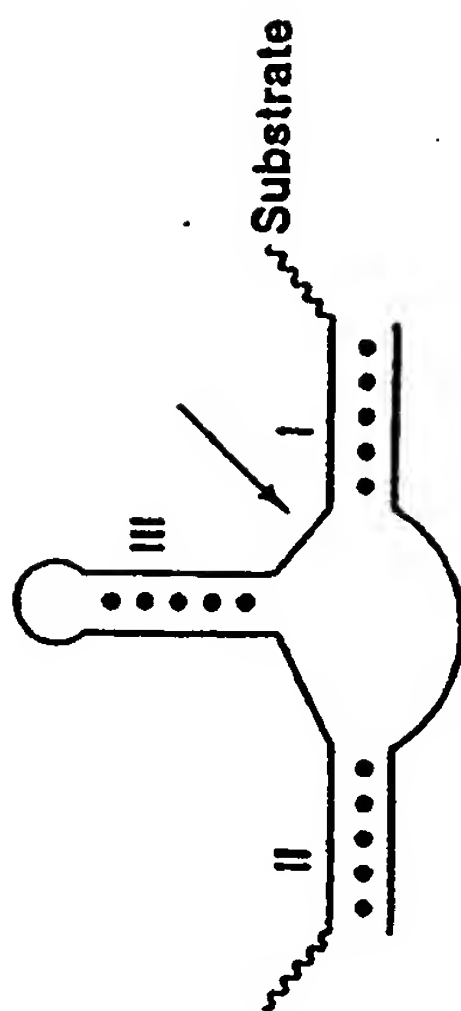
a

FIG. 2c.



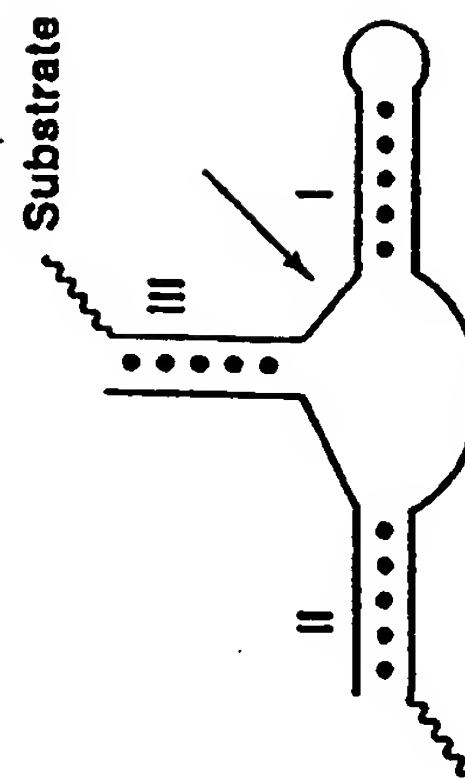
c

FIG. 2b.



b

FIG. 2d.



d

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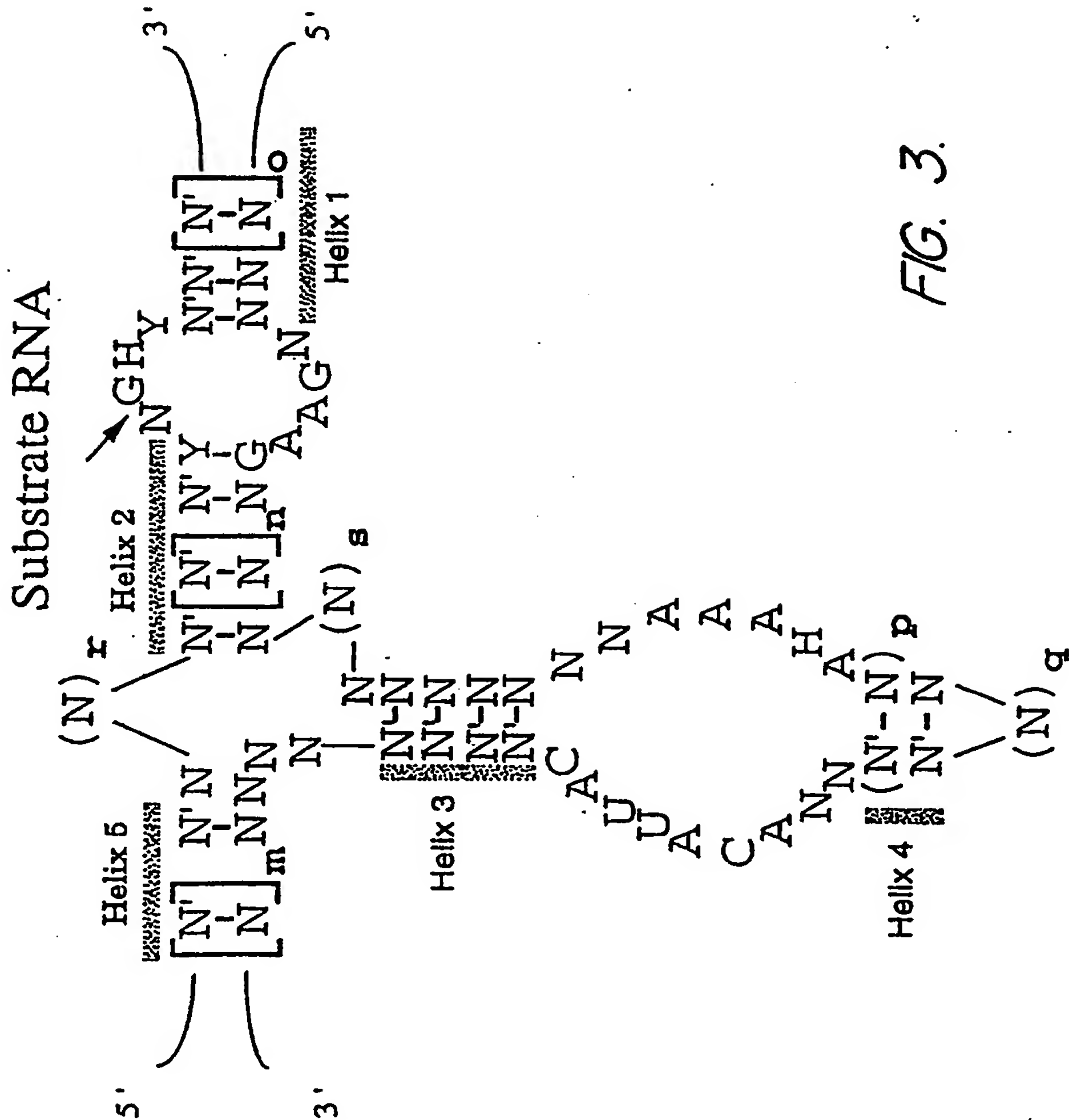
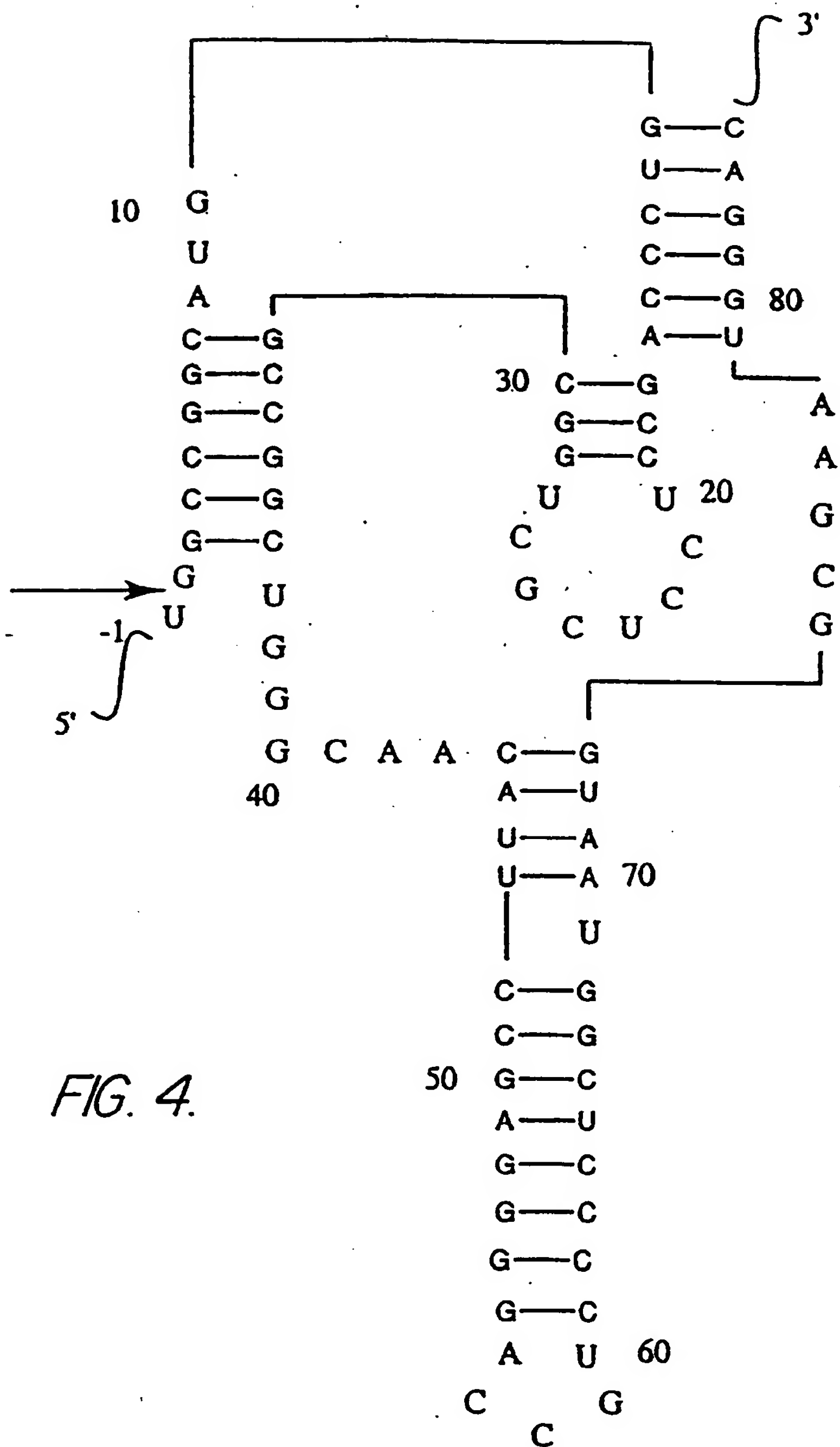


FIG. 3.

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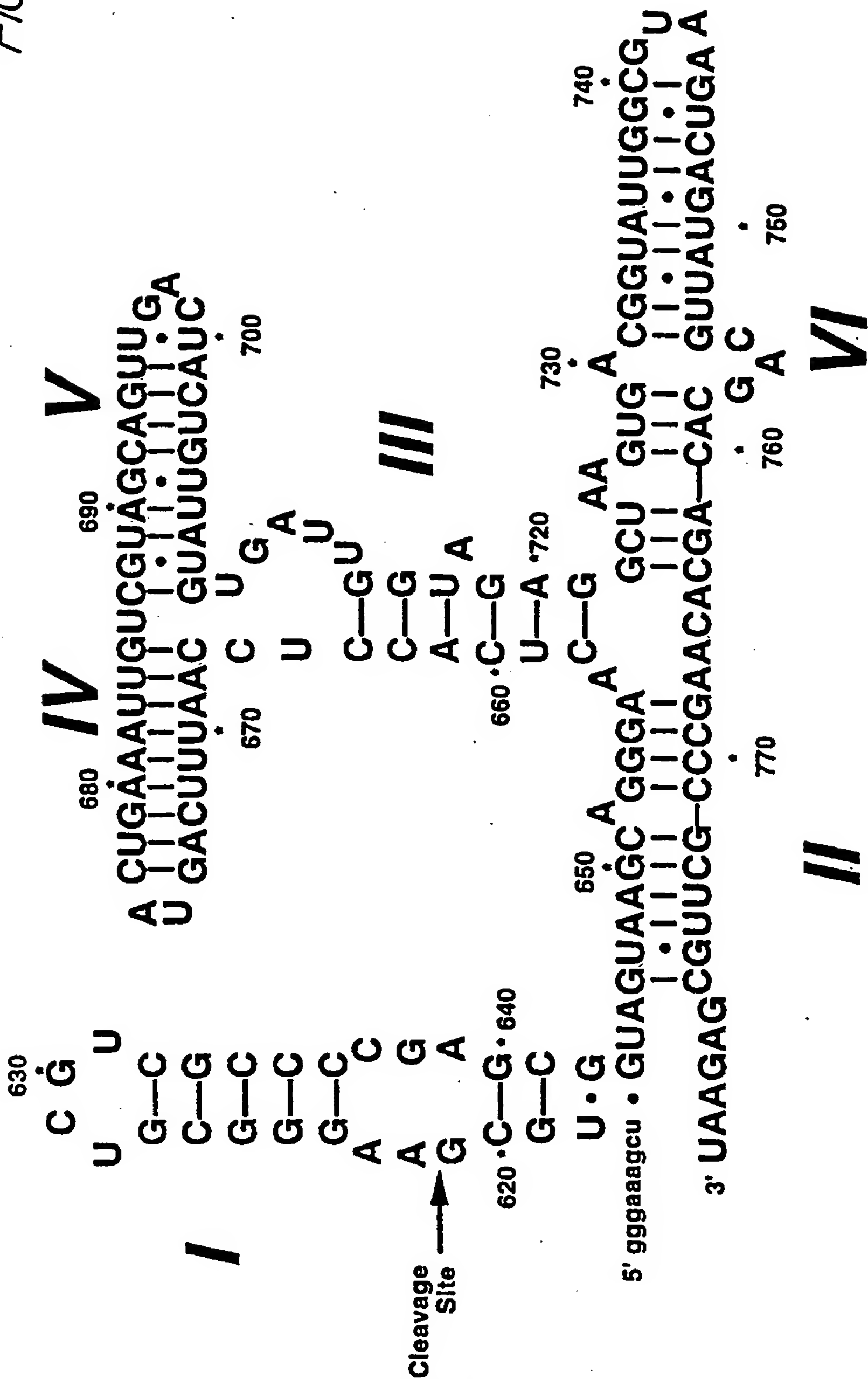


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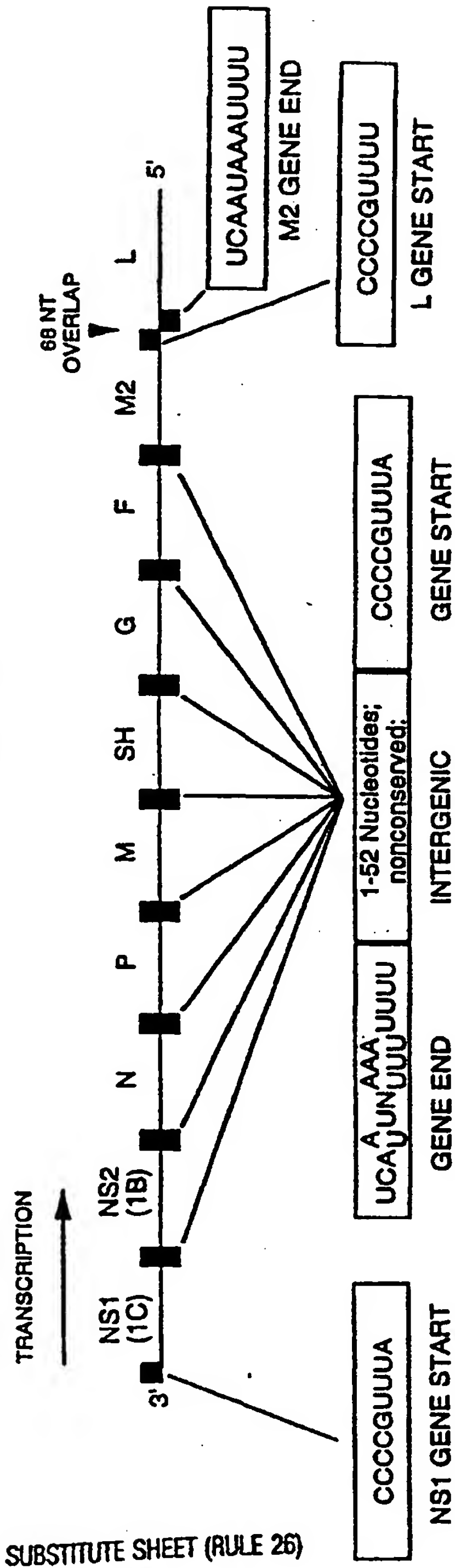
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FIG. 5.



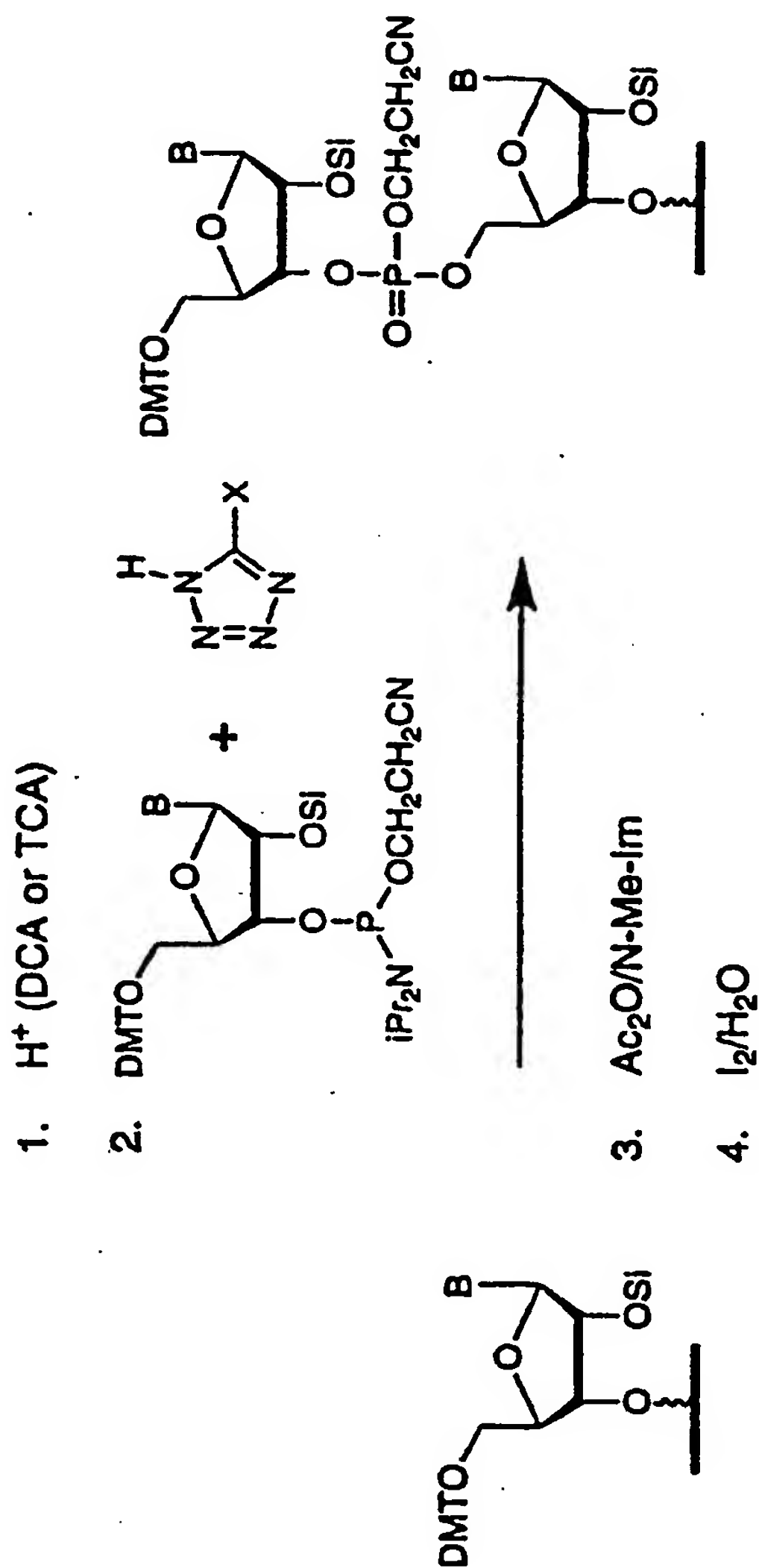
SUBSTITUTE SHEET (RULE 26)

FIG. 6.



Adapted from Virology, Second Edition, Edited by B.N. Fields, 1990.

FIG. 7.



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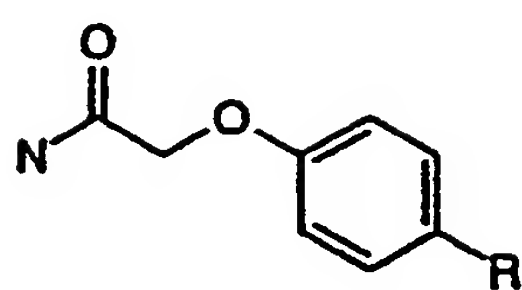
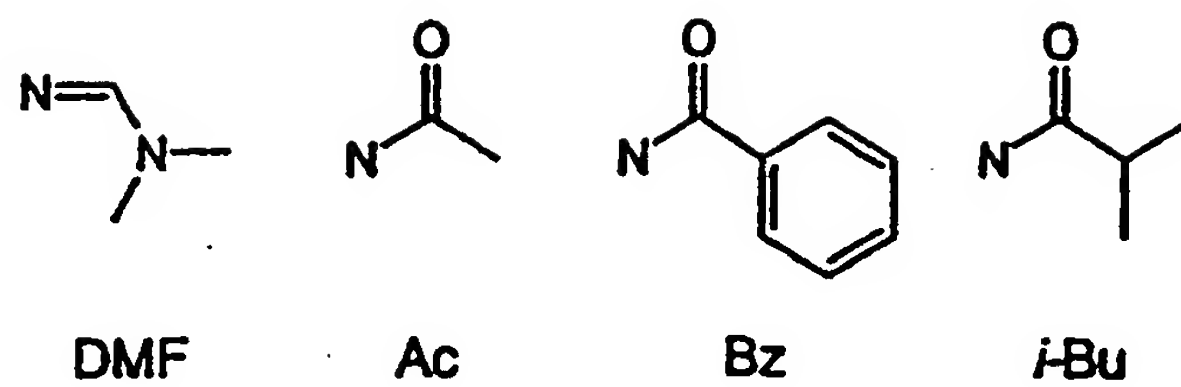


FIG. 8.

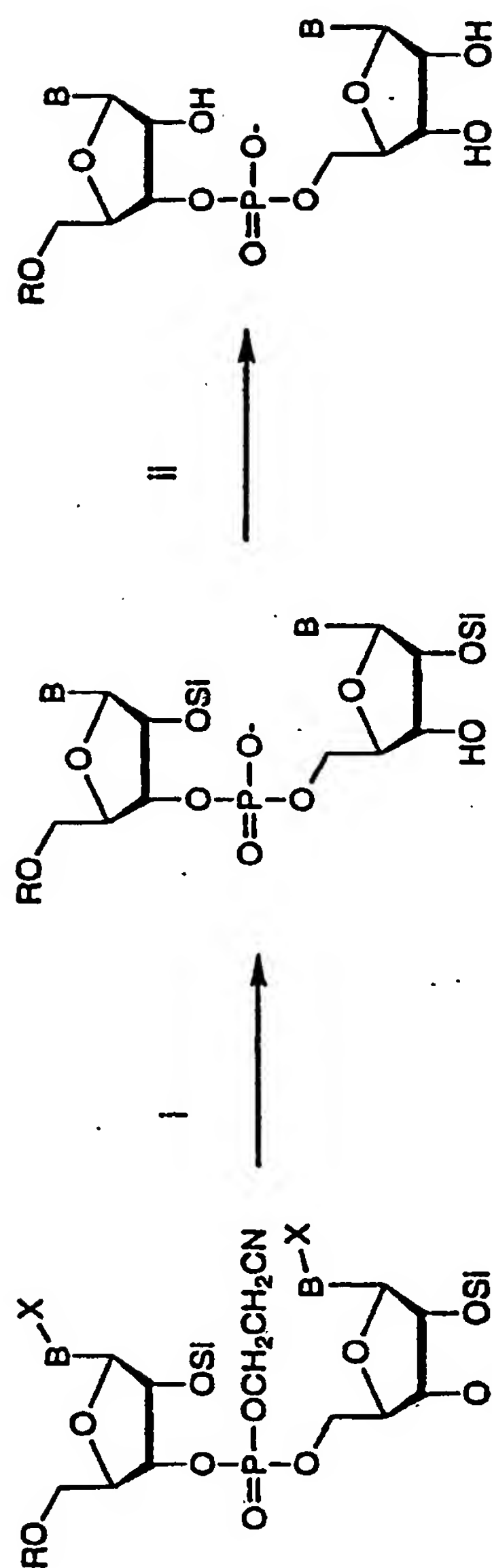
R = H = PAC

R = tBu = TAC

R = iPr = iPPAC

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FIG. 9.



I MA or AMA, 30 m @ 65 °C

II anhydrous TEA·HF, 30 m @ 65 °C

III H^+

R = H or DMT or other hydroxyl protection

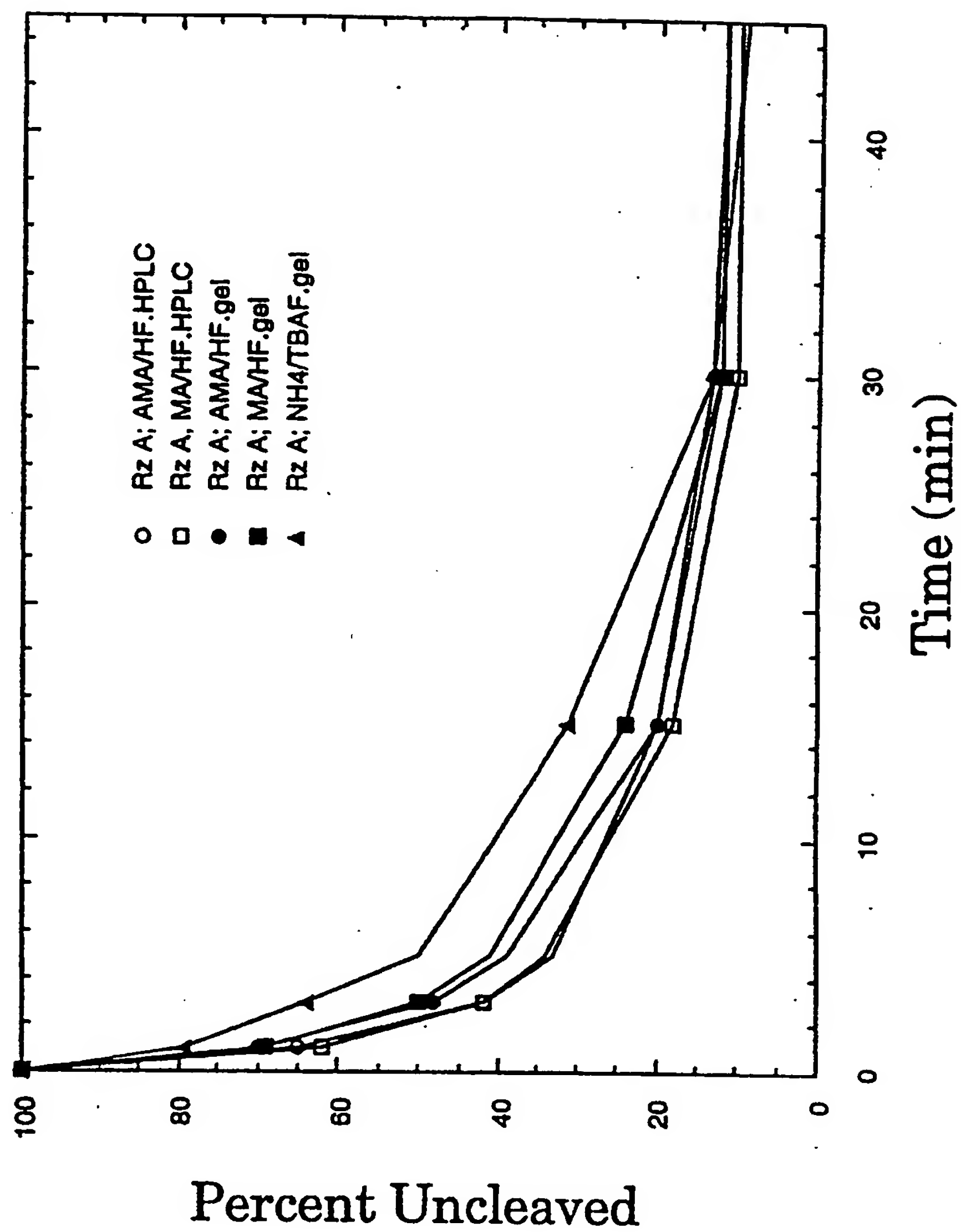
X = Exocyclic Amino protection

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FIG. 10.

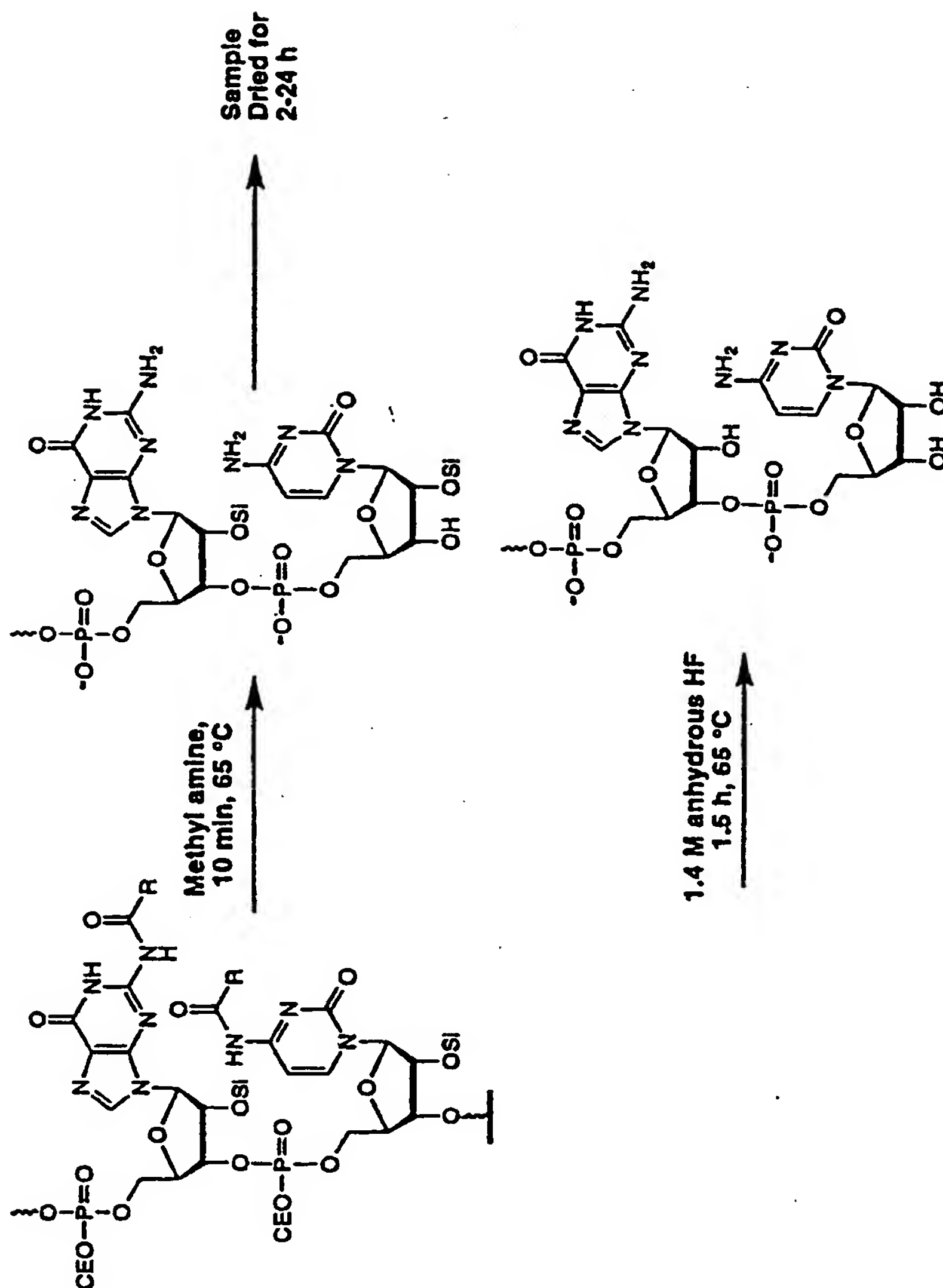


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FIG. 11.



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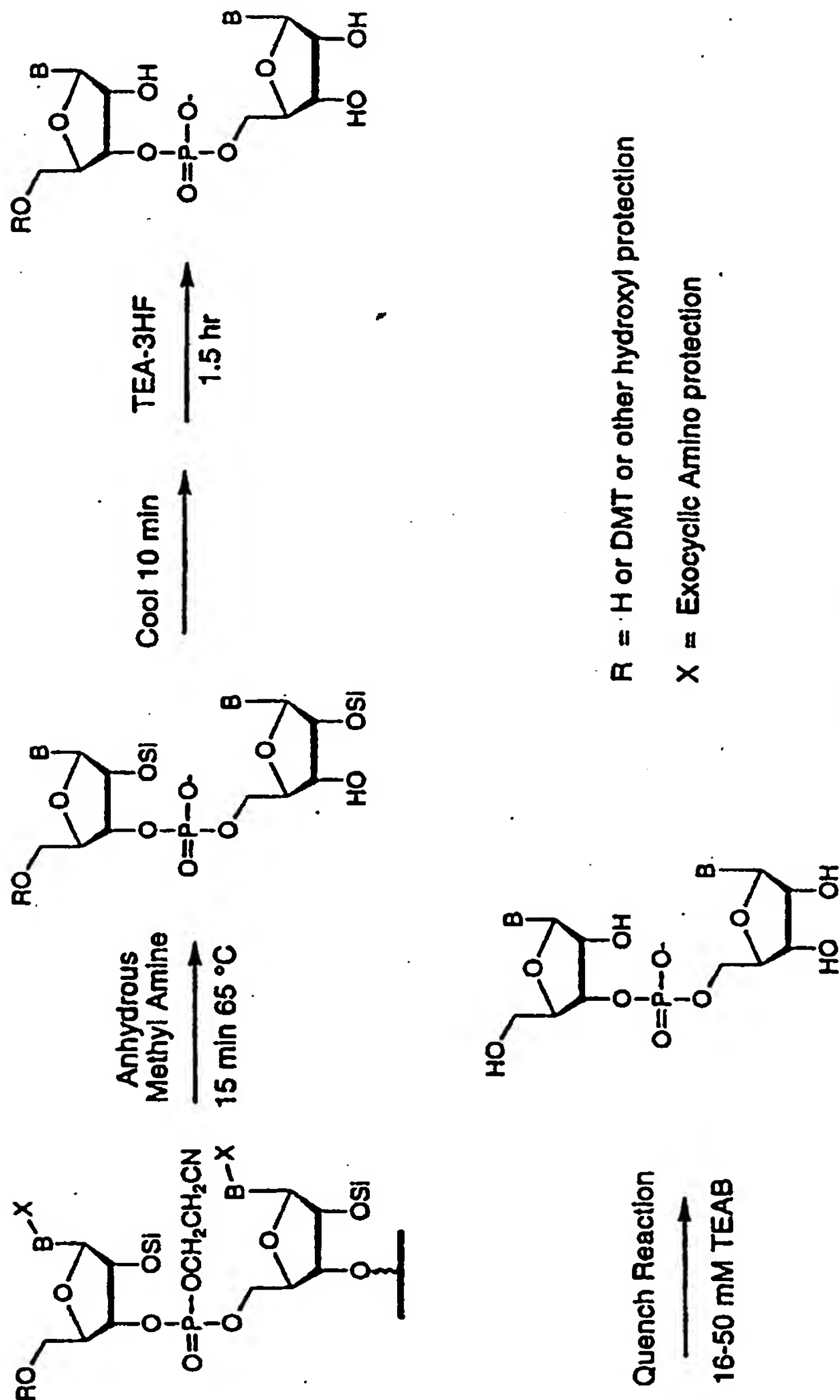


FIG. 12.

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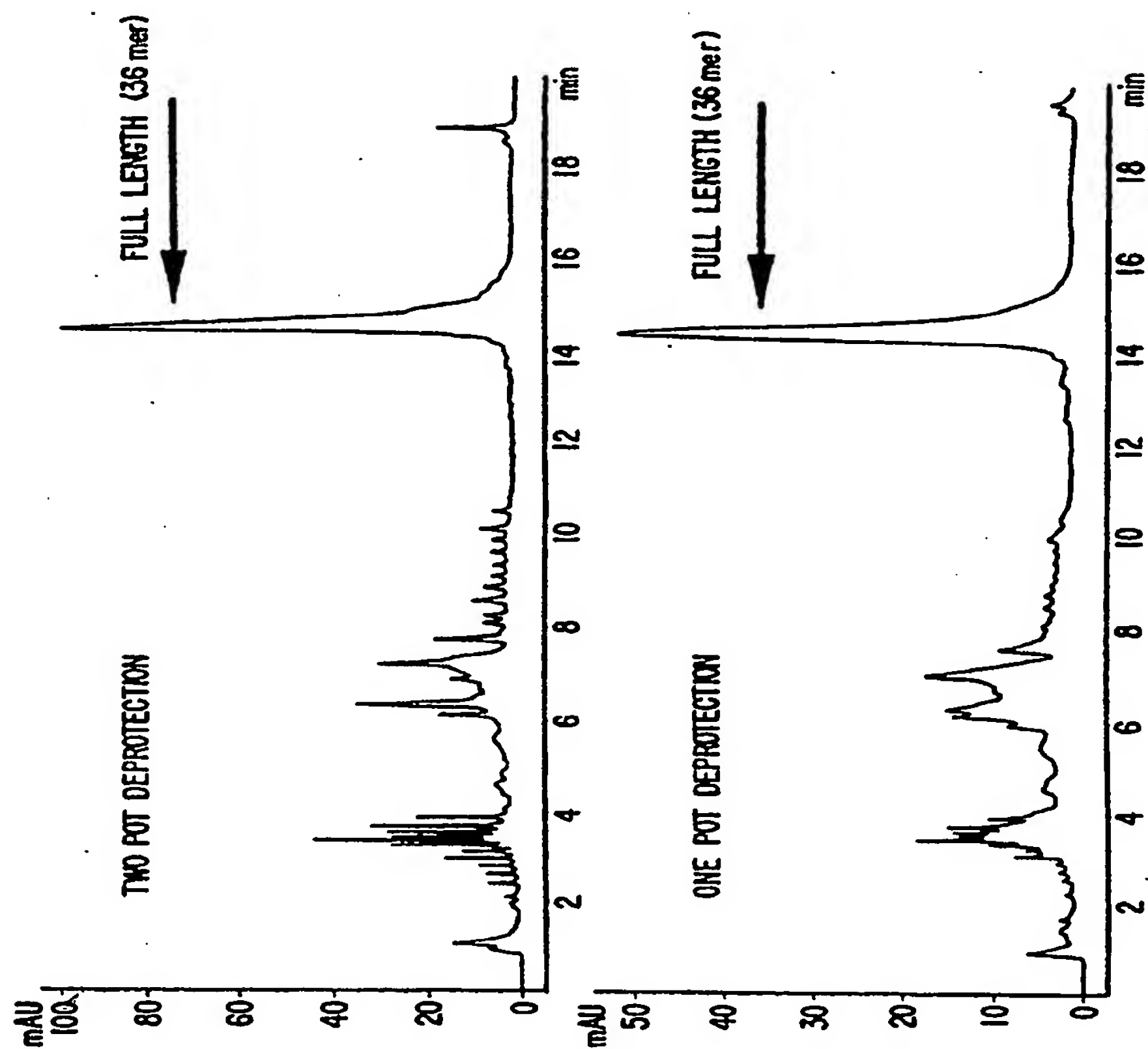


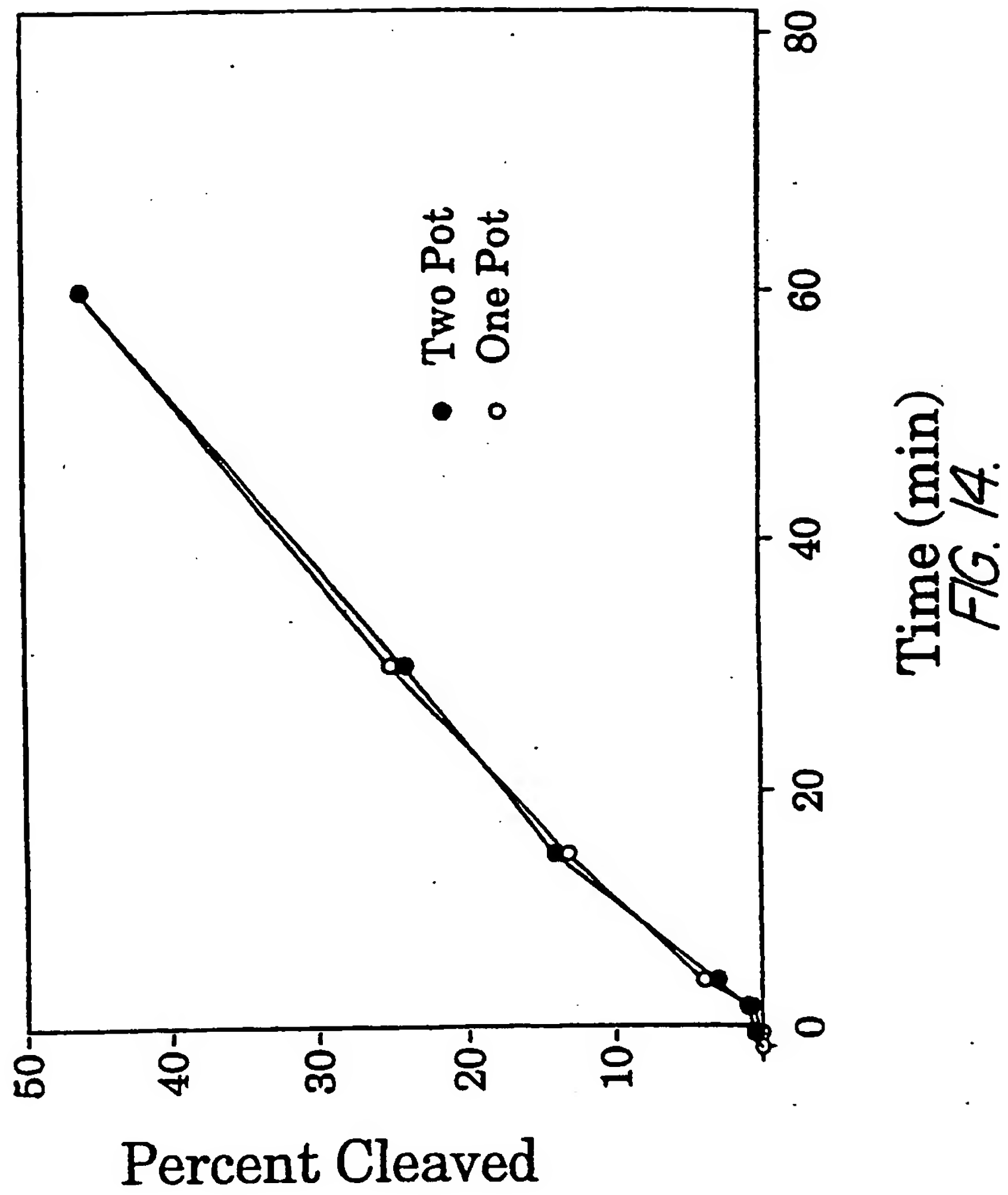
FIG. 13a.

FIG. 13b.

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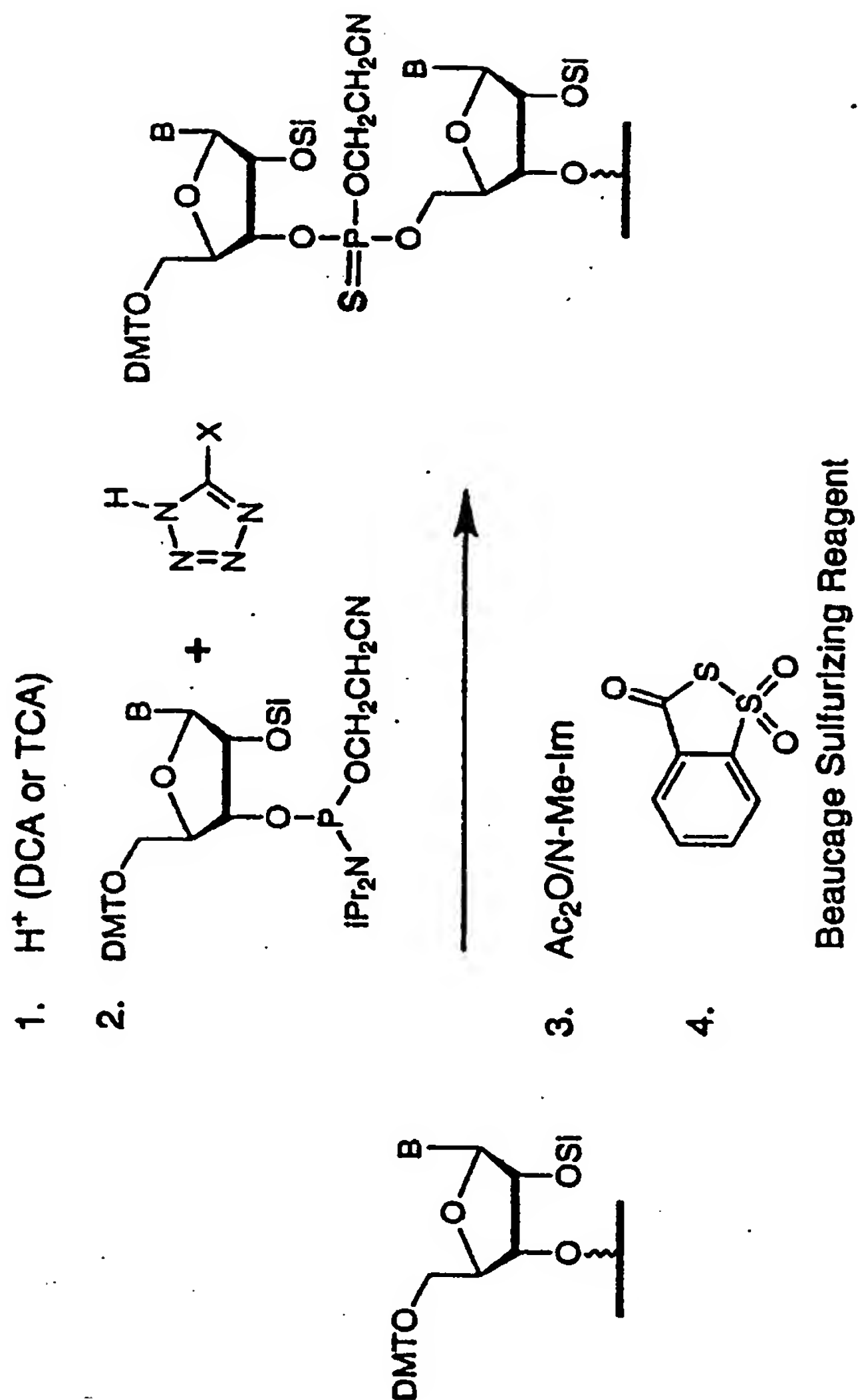


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NUC 37921

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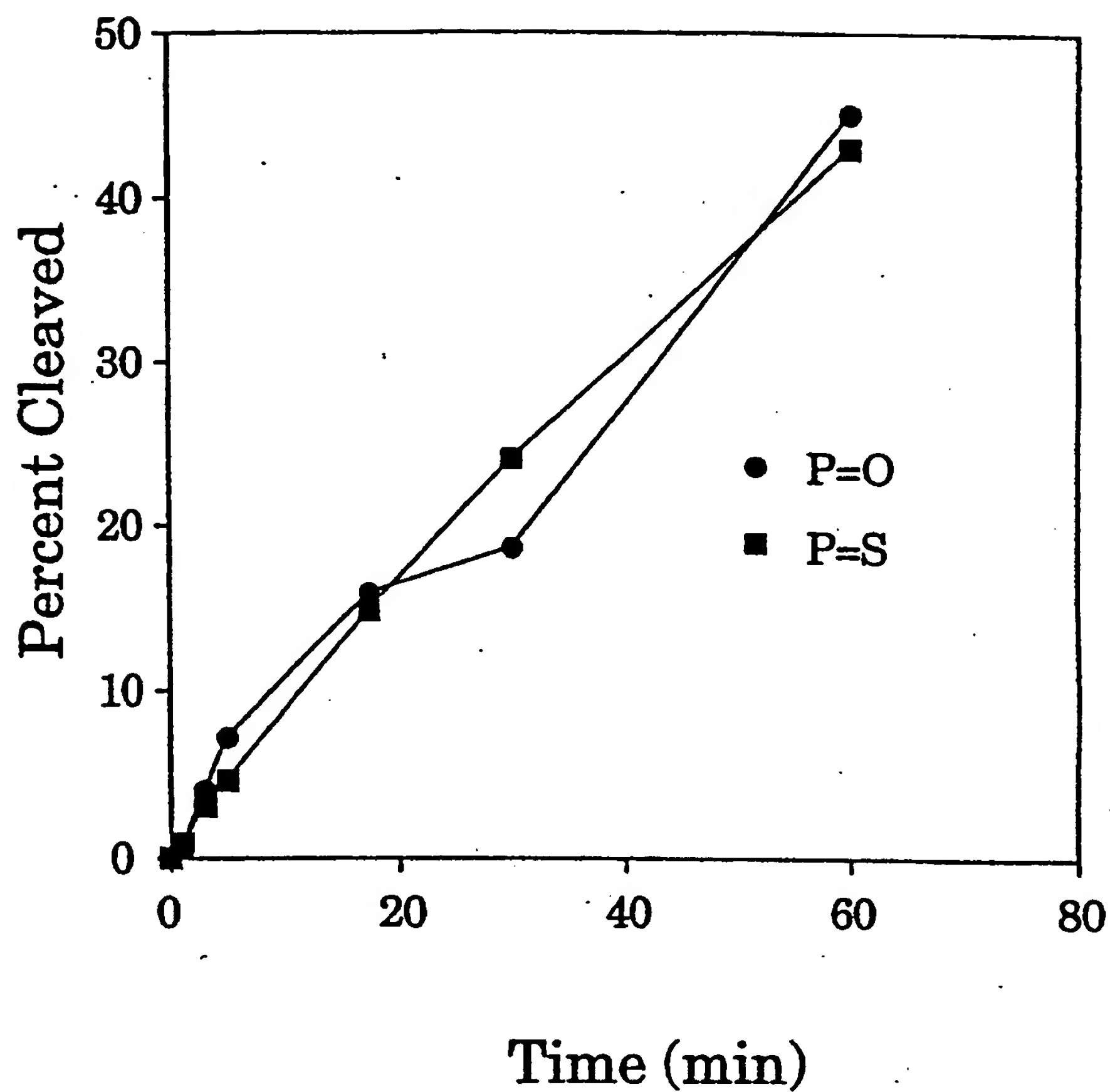
FIG. 15.



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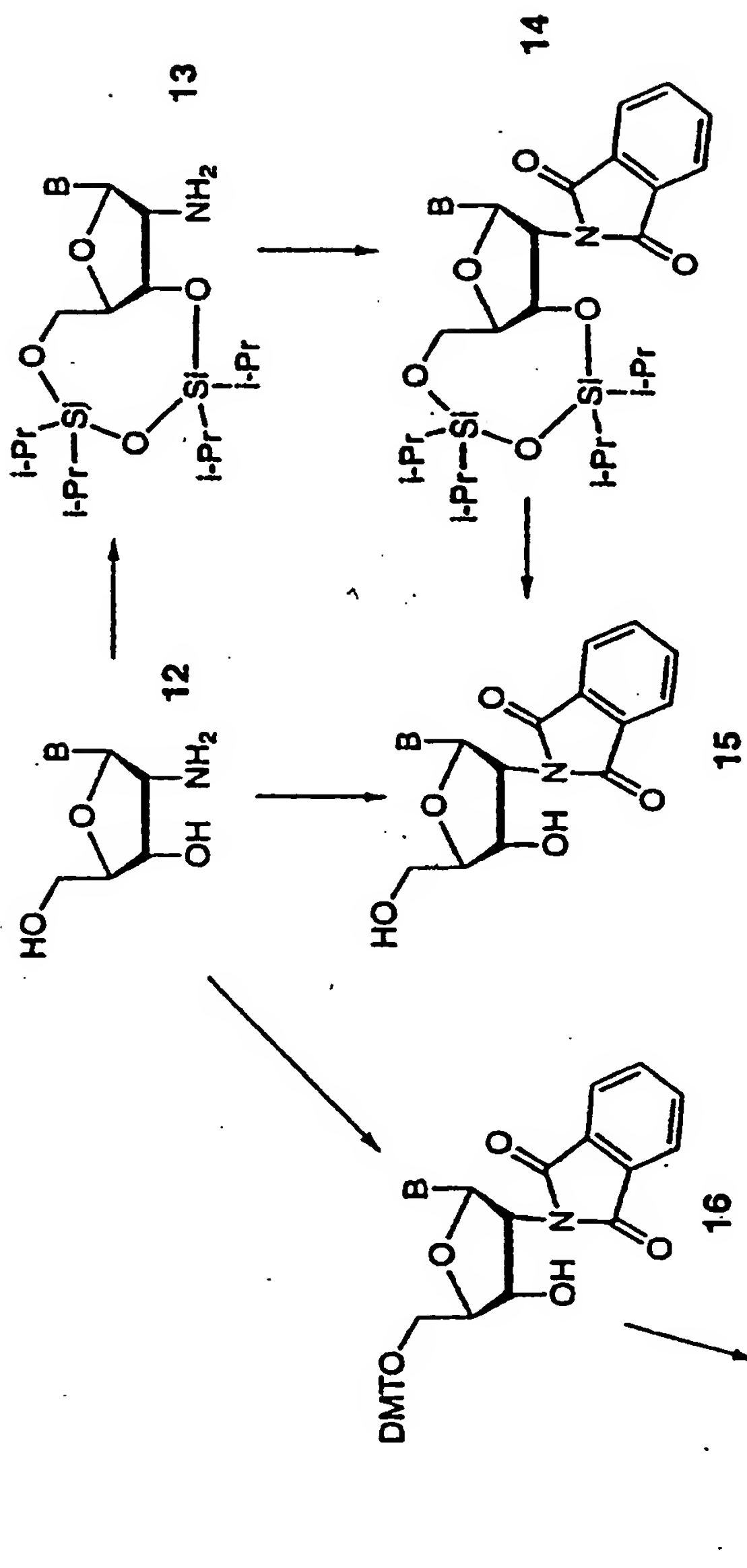


Time (min)
FIG. 16.

SUBSTITUTE SHEET (RULE 26)

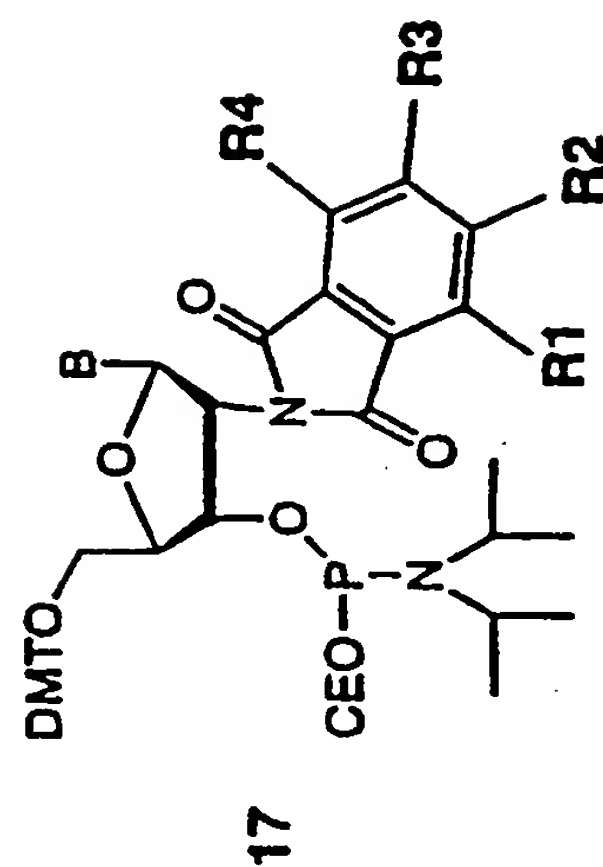
NUC 37923

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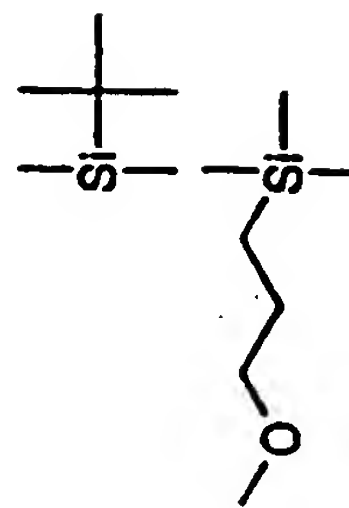
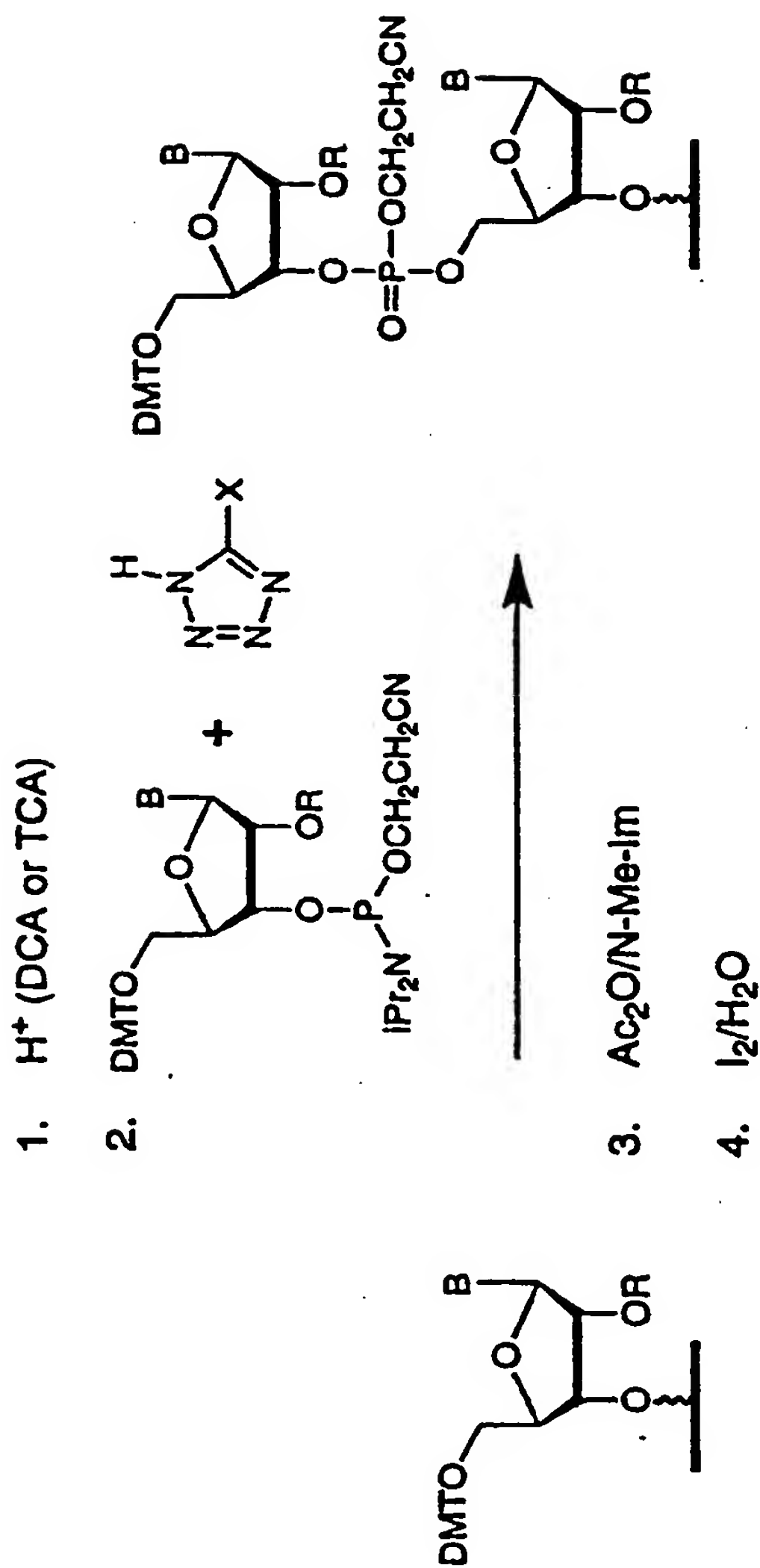
B = any regular or modified base or abasic
R1-R4 = alkyl or halogen

FIG. 17.



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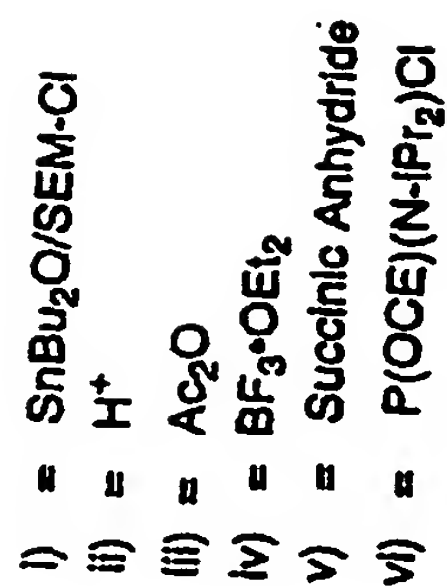
FIG. 18.



R = Silyl ether (prior art)

(trimethylsilyl)ethoxymethyl (SEM)

FIG. 19.

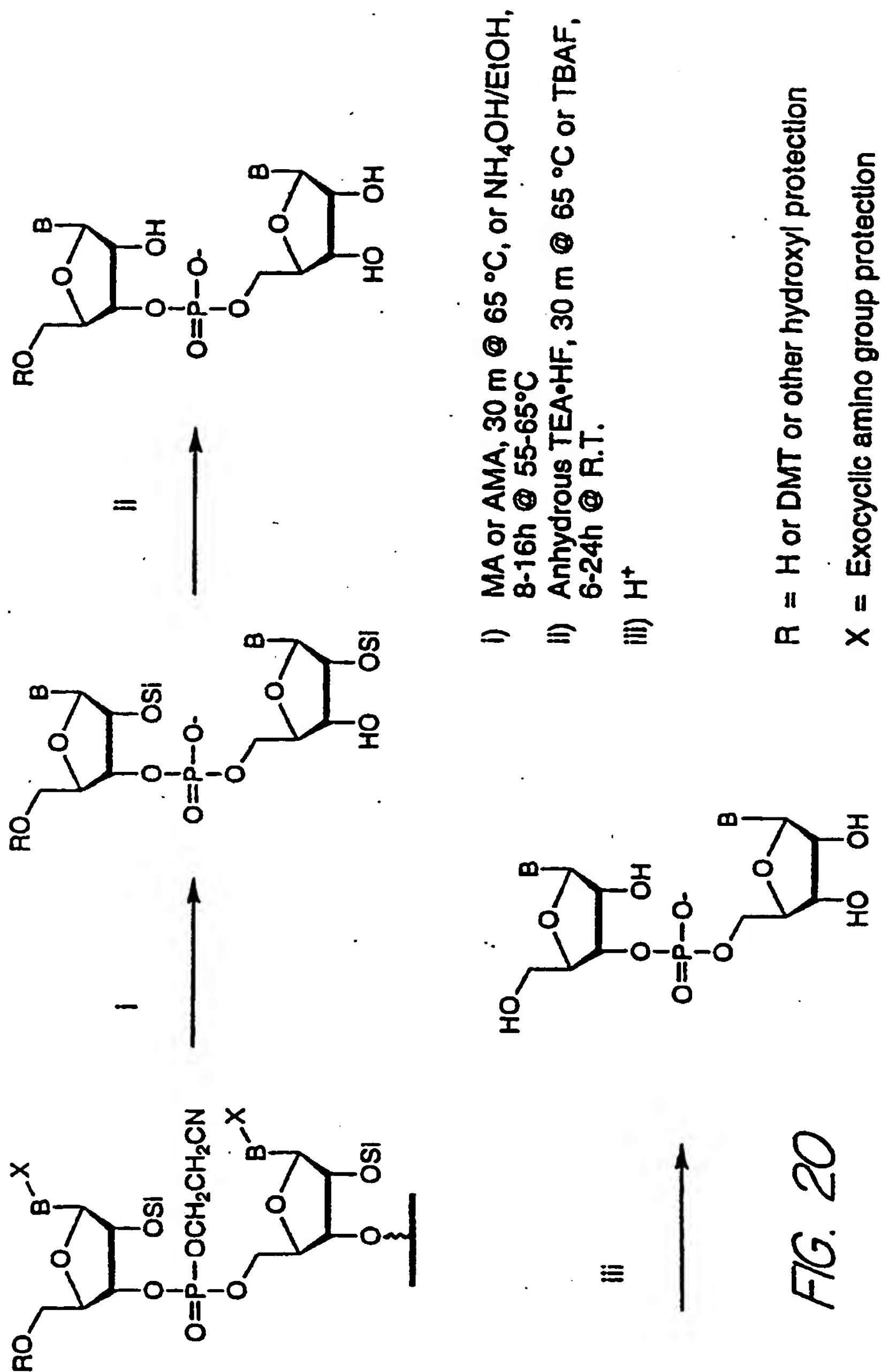


B = Protected A, C, G, U, T, 2AP, I, DIAP, P etc.

SEM = (trimethylsilyl)ethoxymethyl!

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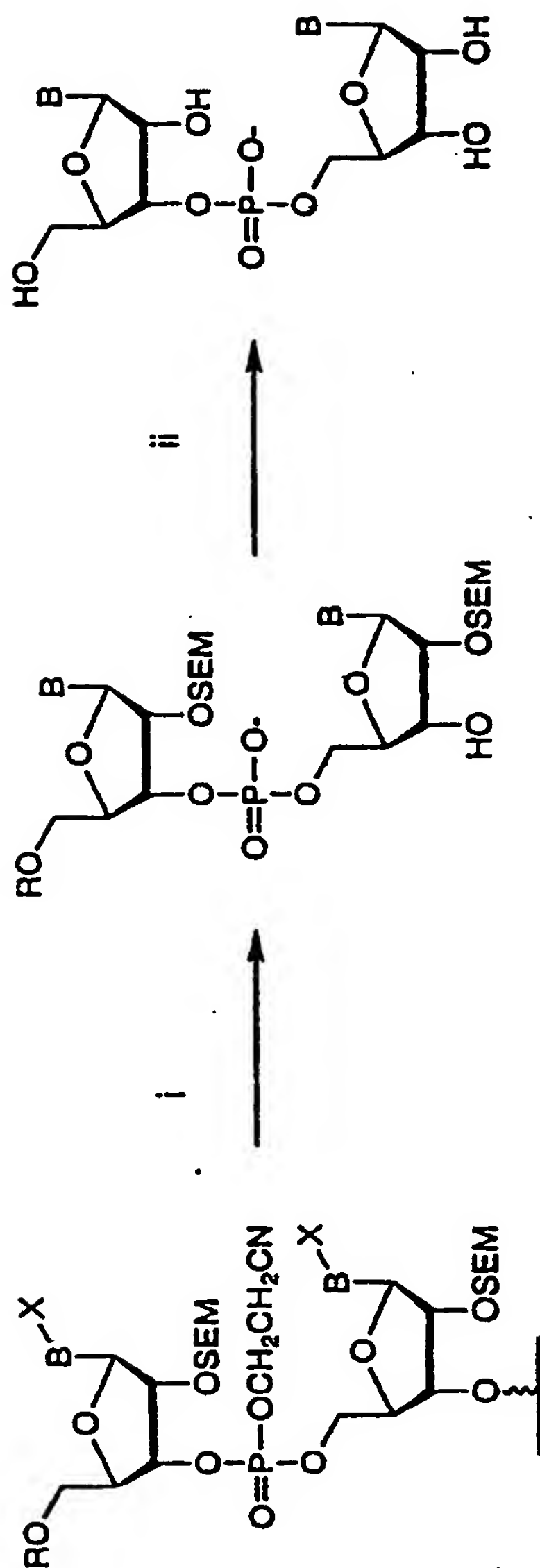
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FIG. 21.

i) MA or AMA, 30 m @ 65 °C or NH₄OH or NH₄OH/EtOH, 8-16h @ 55-65°Cii) BF₃•OEt₂

SEM = (trimethylsilyl)ethoxymethyl

R = H or DMT or other hydroxyl protection

X = Exocyclic amino group protection

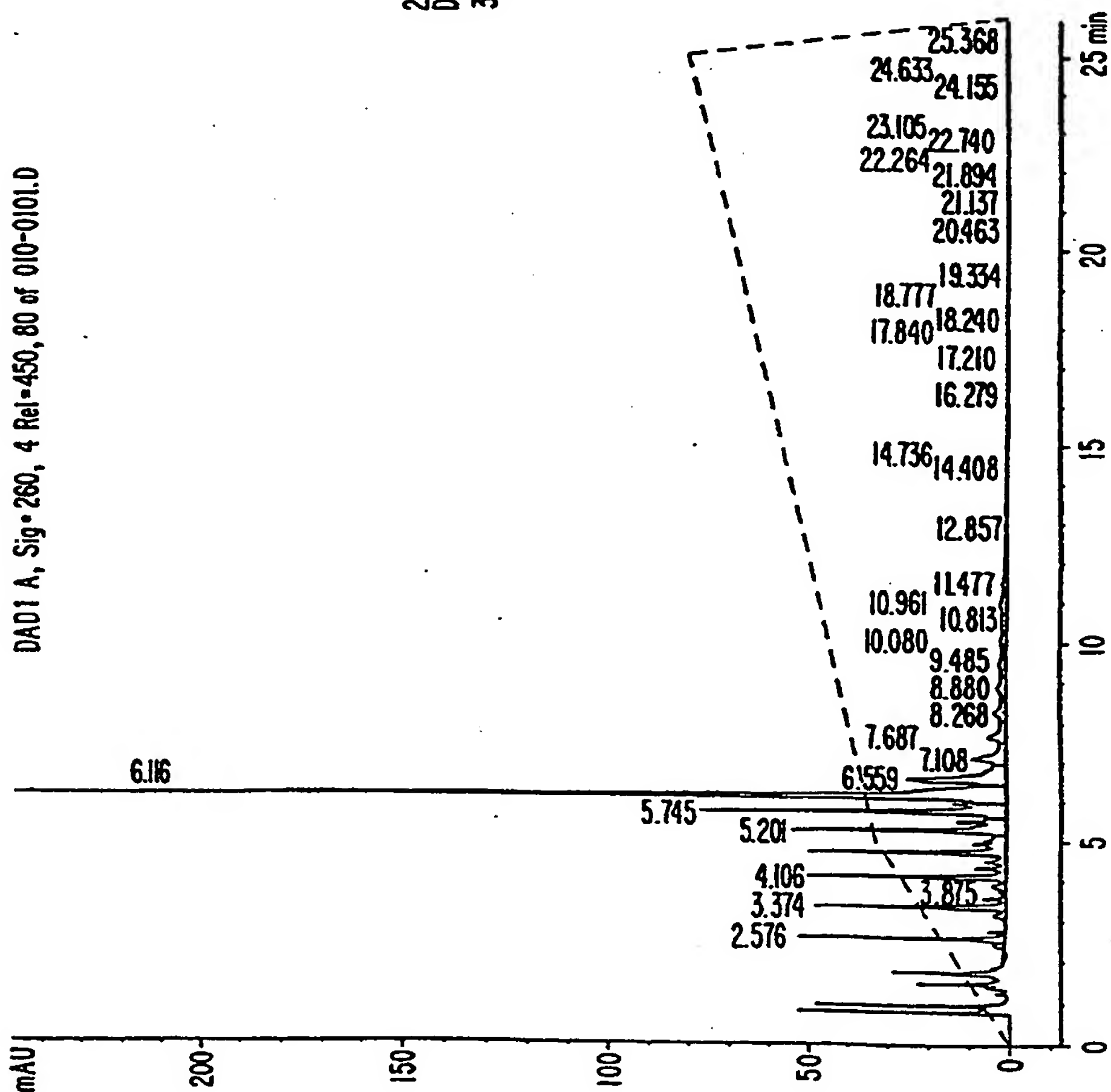
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FIG. 22.

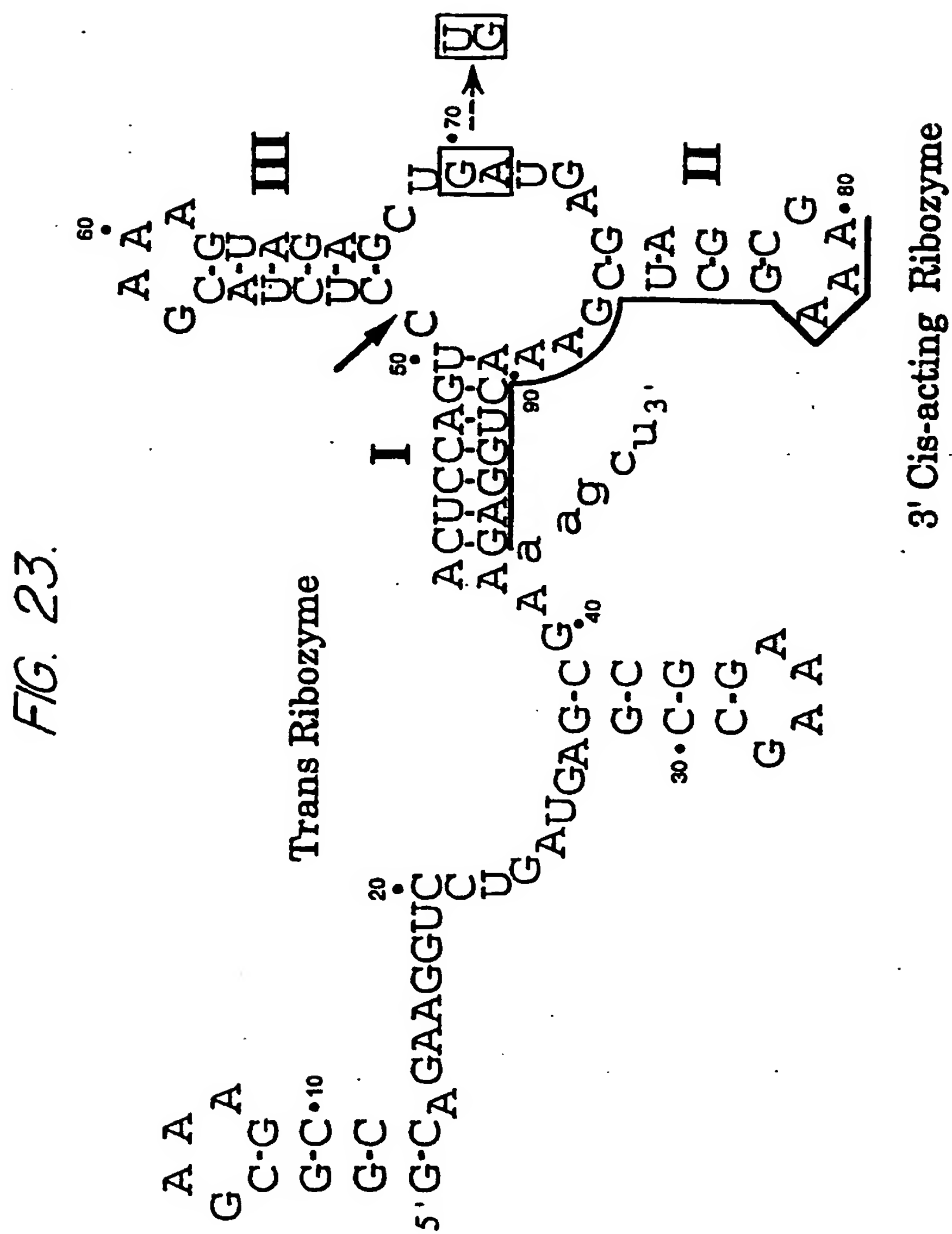
2'-O-SEM PROTECTED U 10-mer
DEPROTECTED WITH $\text{BF}_3 \cdot \text{OEt}_2$
30m, 3eq./nucleotide



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NUC 37929

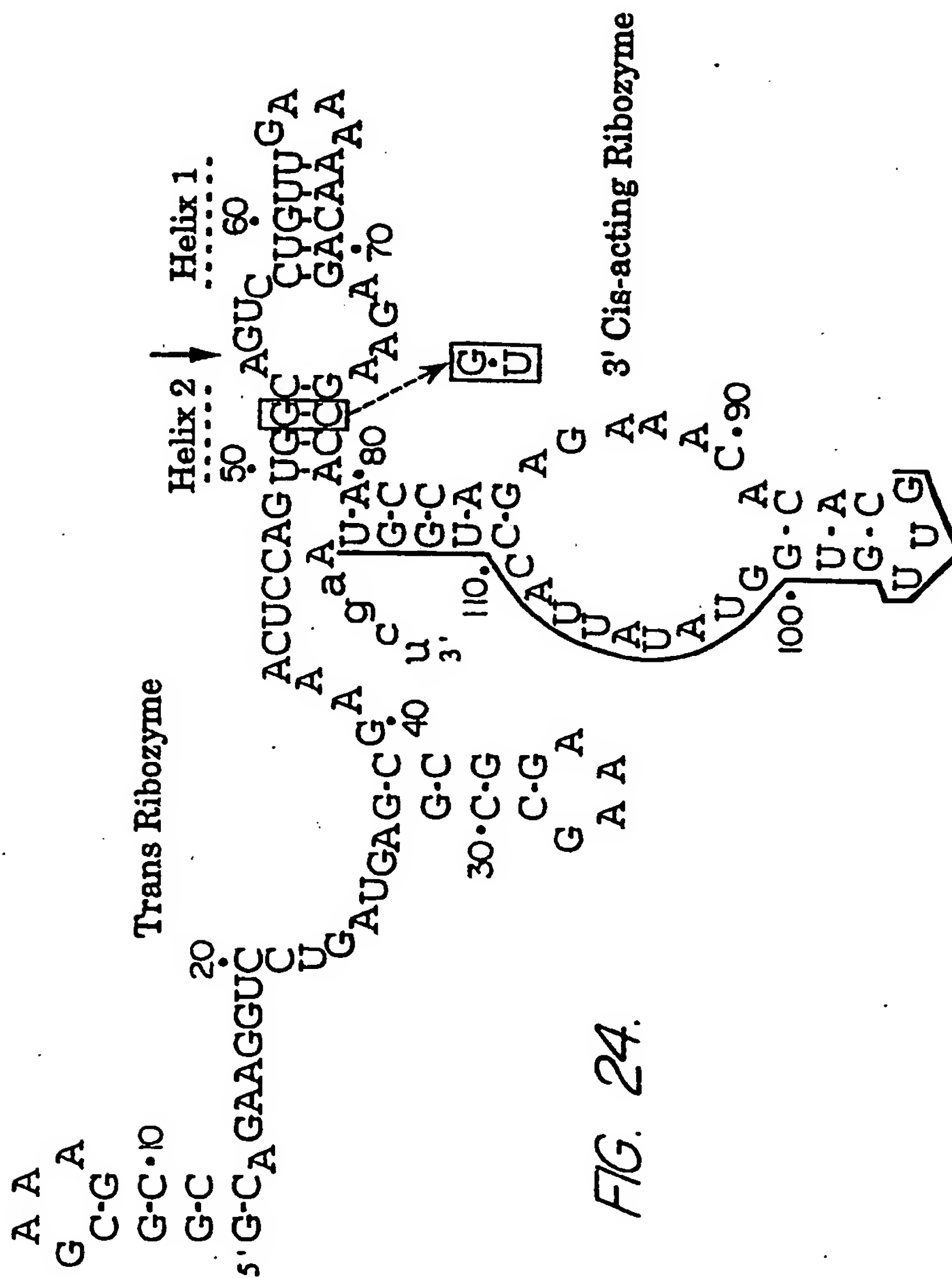
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SUBSTITUTE SHEET (RULE 26)

NUC 37930

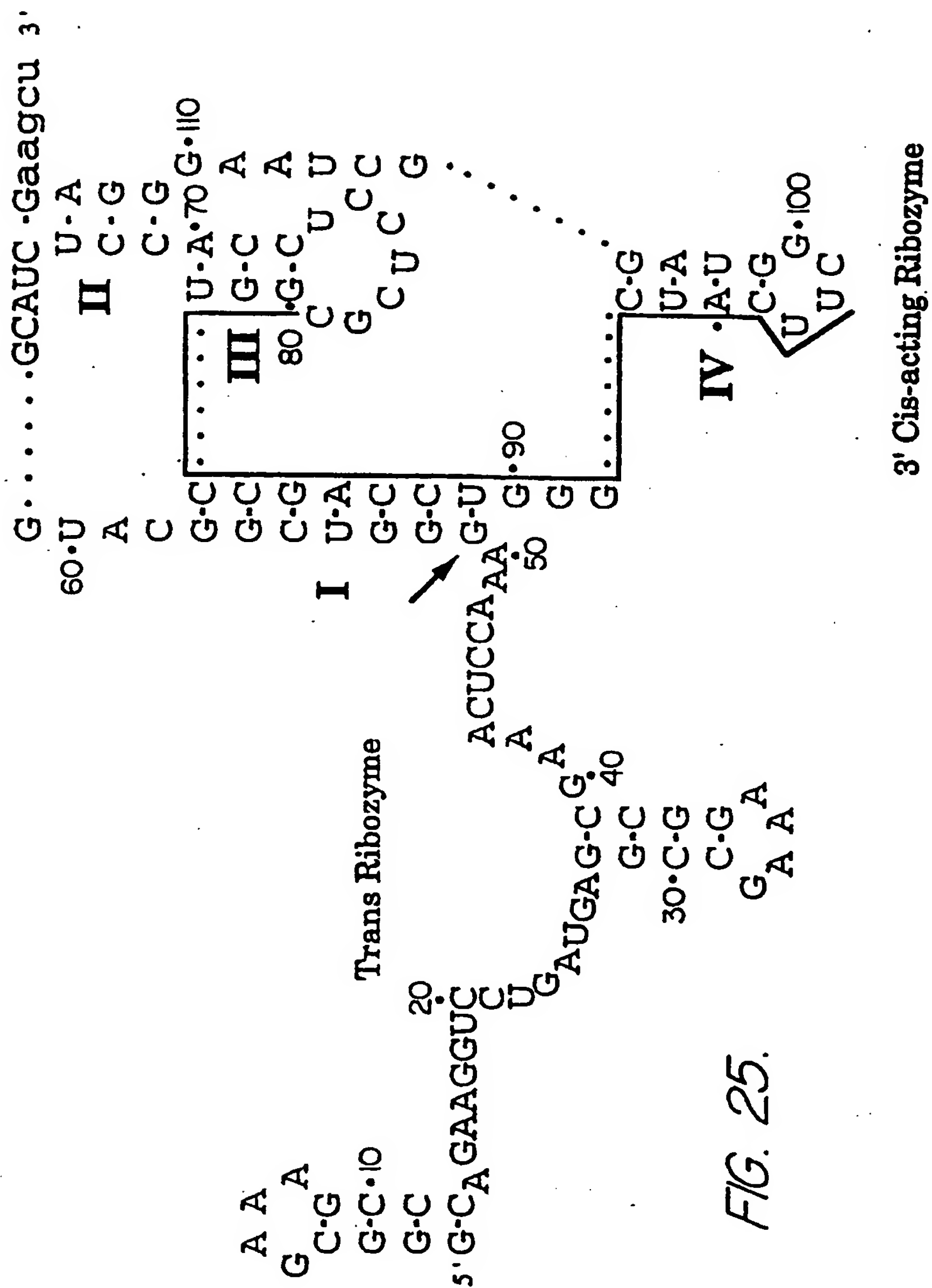
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NUC 37931

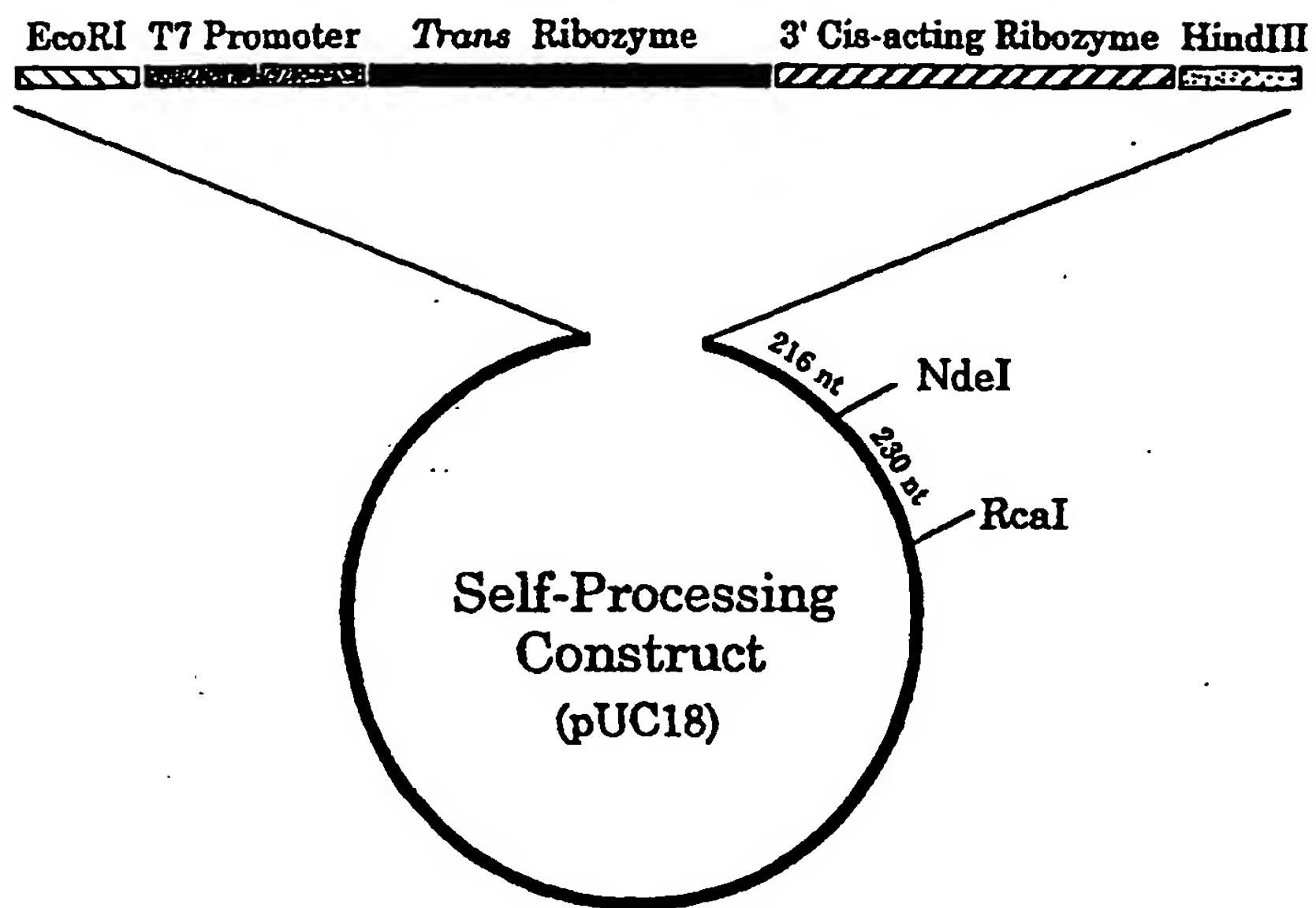
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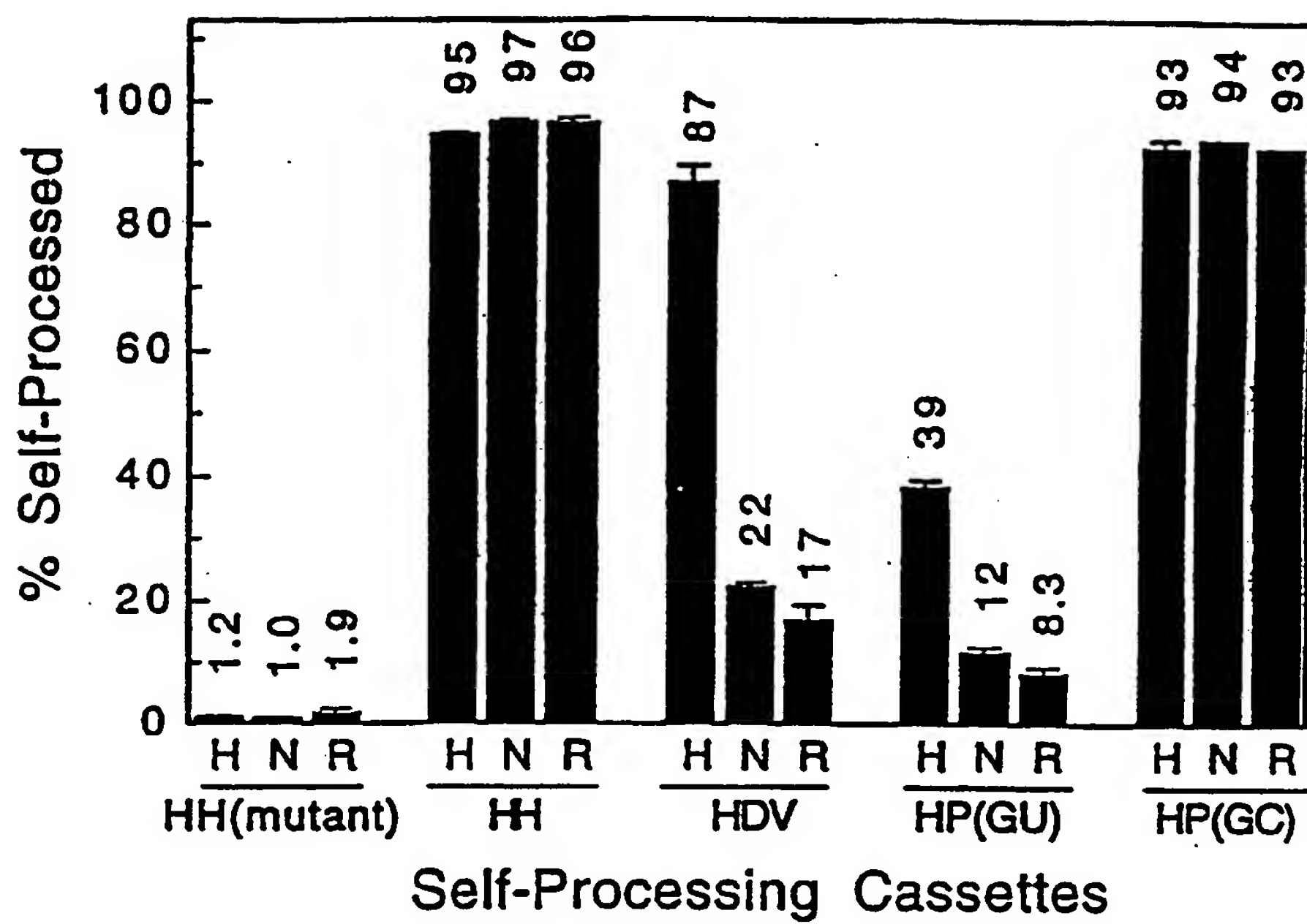
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FIG. 26.



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FIG. 27.



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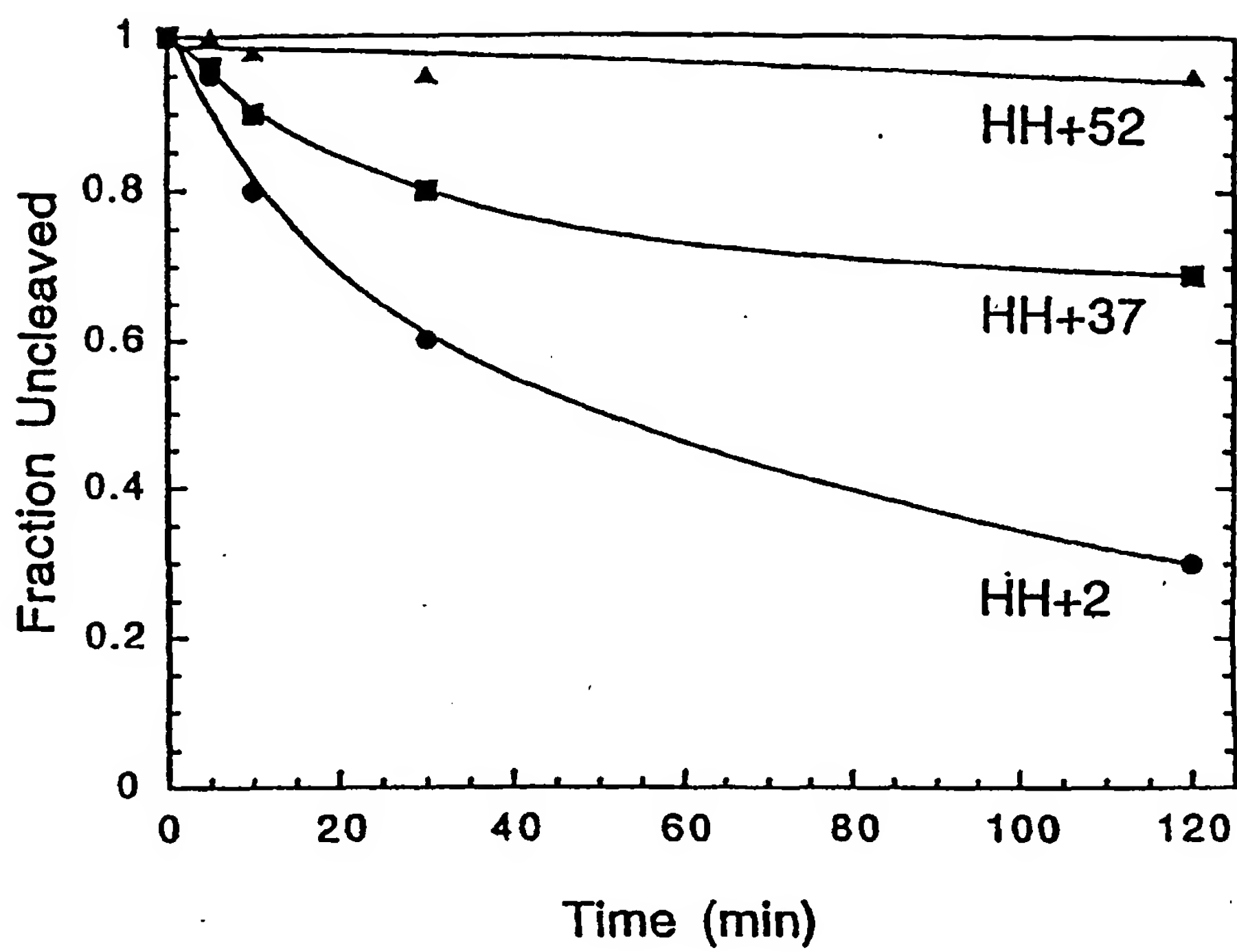
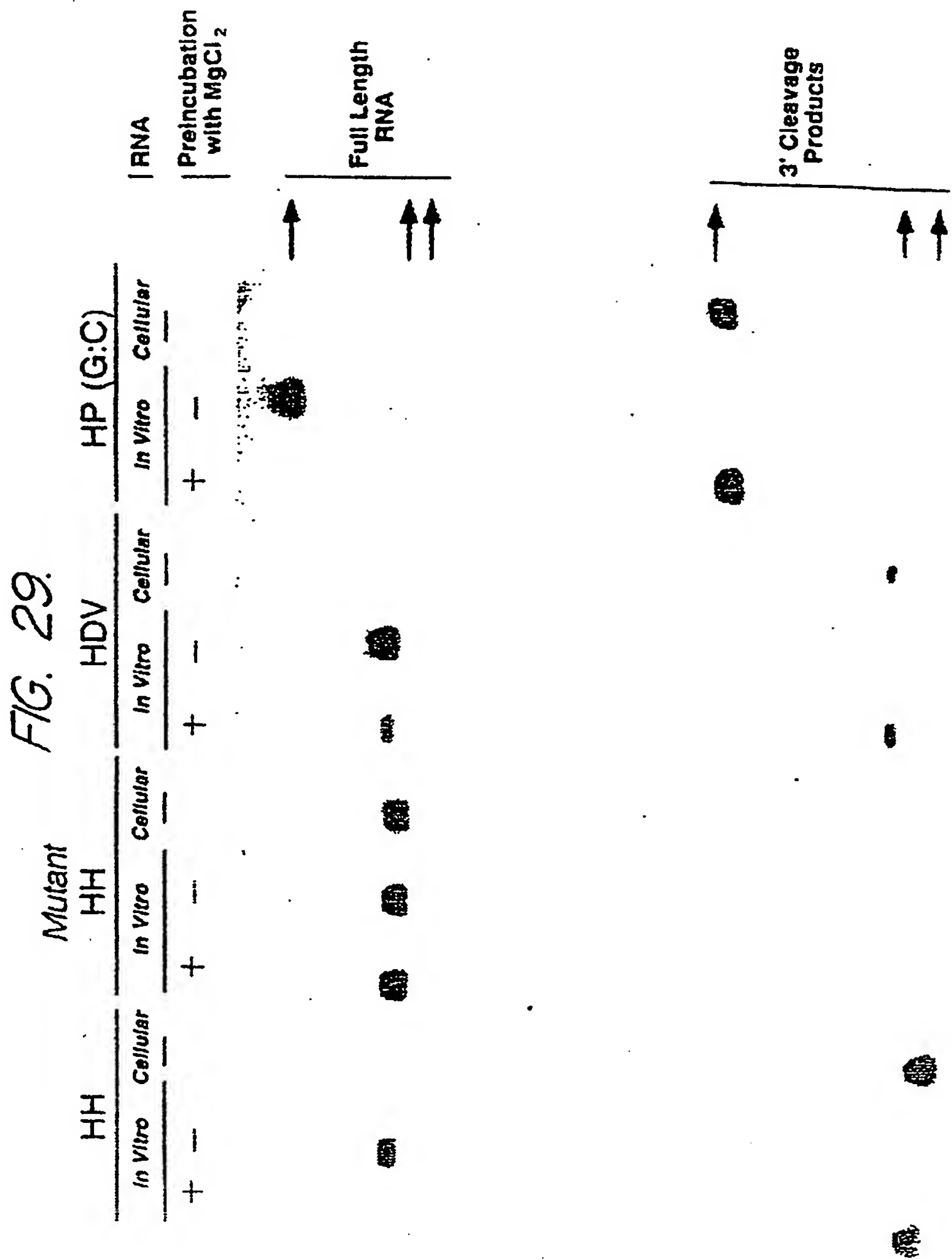


FIG. 28.

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FIG. 30

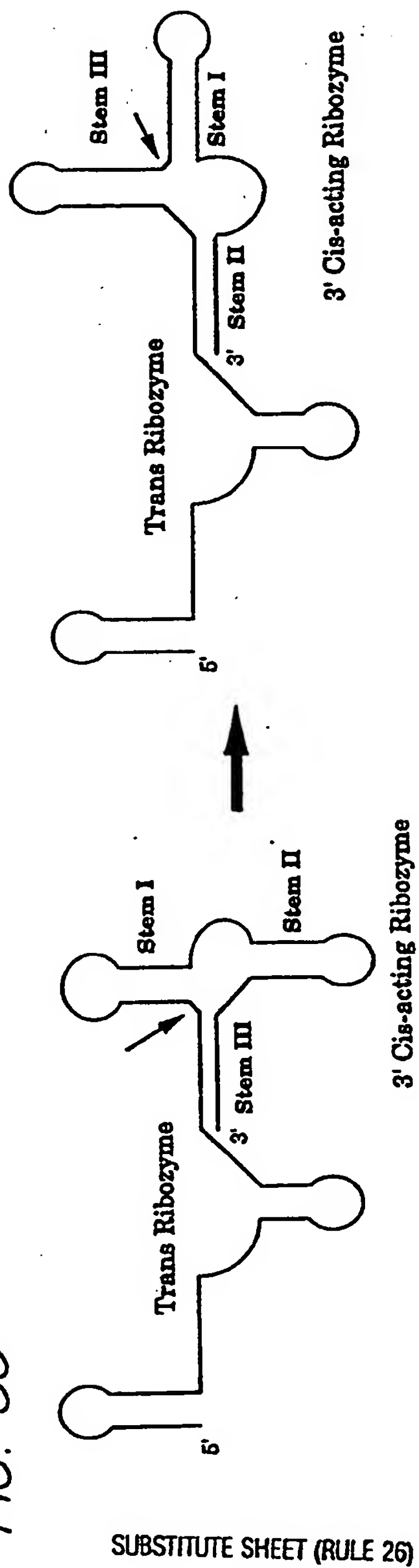
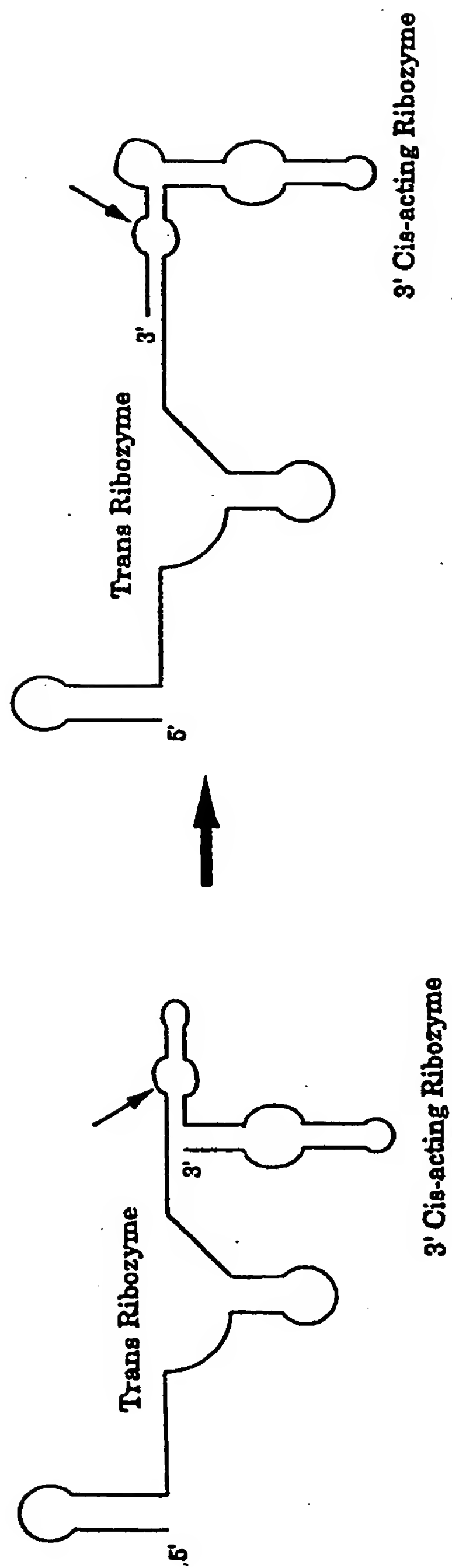


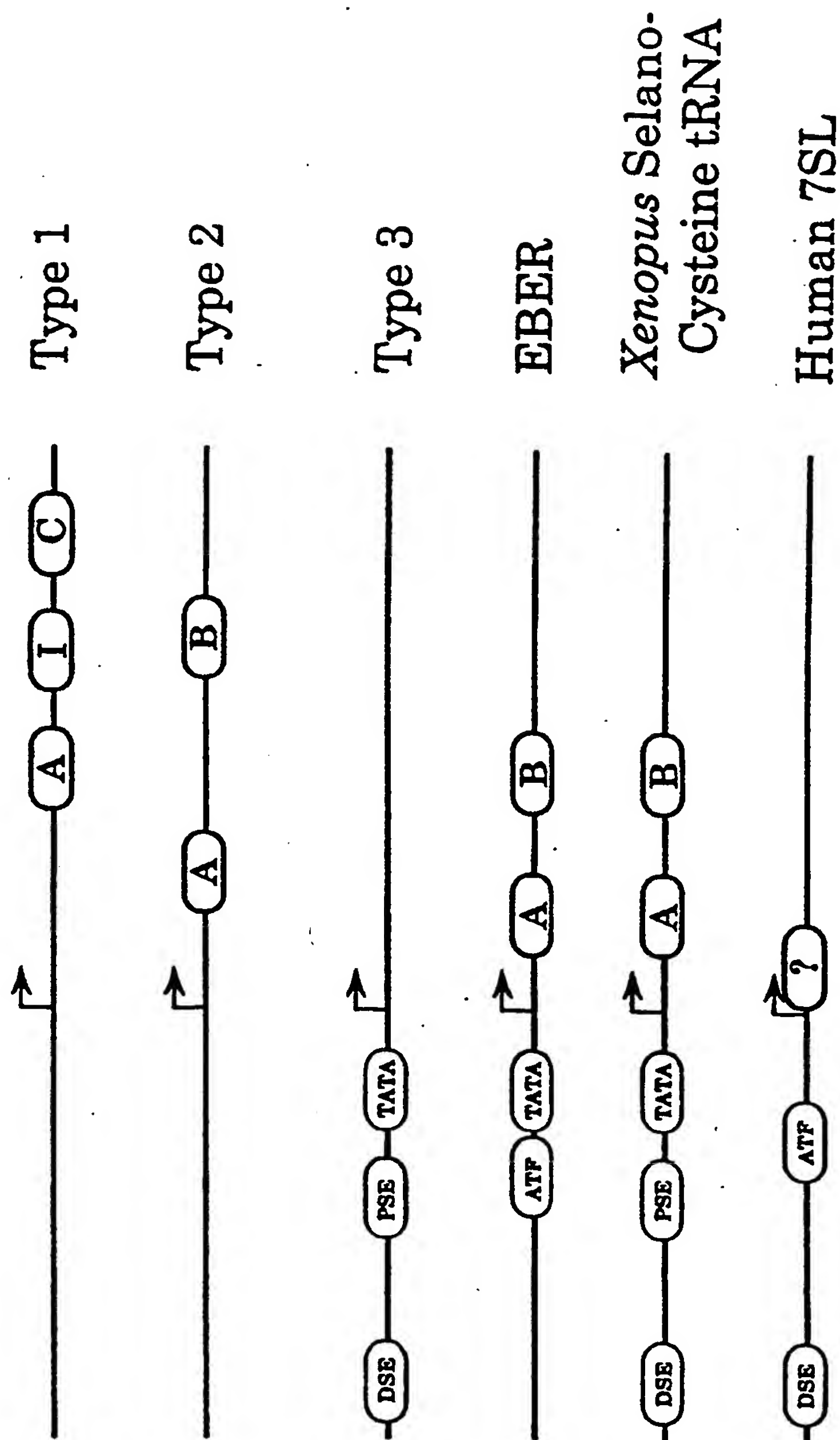
FIG. 31.



SUBSTITUTE SHEET (RULE 26)

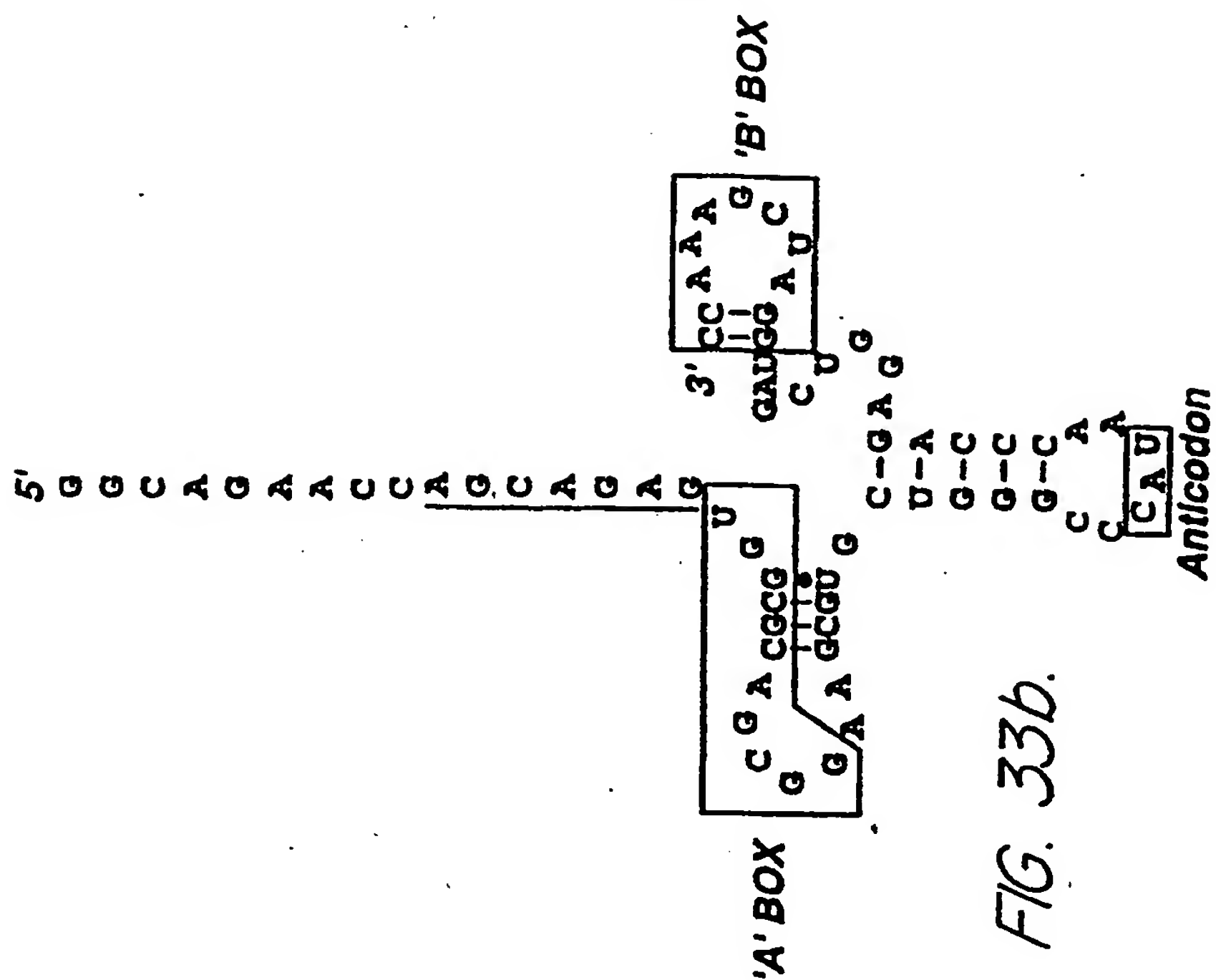
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FIG. 32.

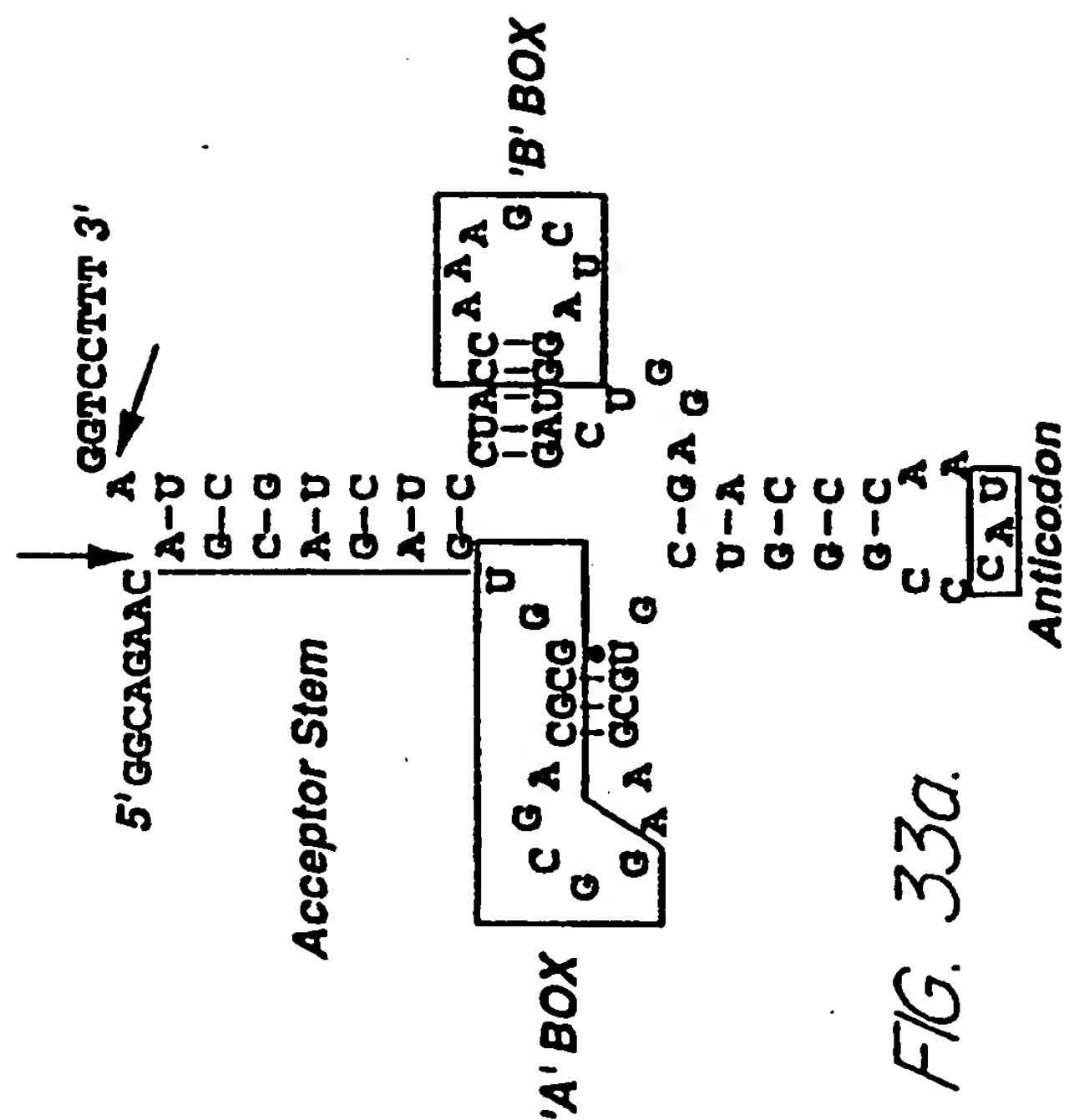


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Δ 3-5



**met
tRNA_i**

FIG. 33a.

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FIG. 34a.
 $\Delta 3-5$ /HHI

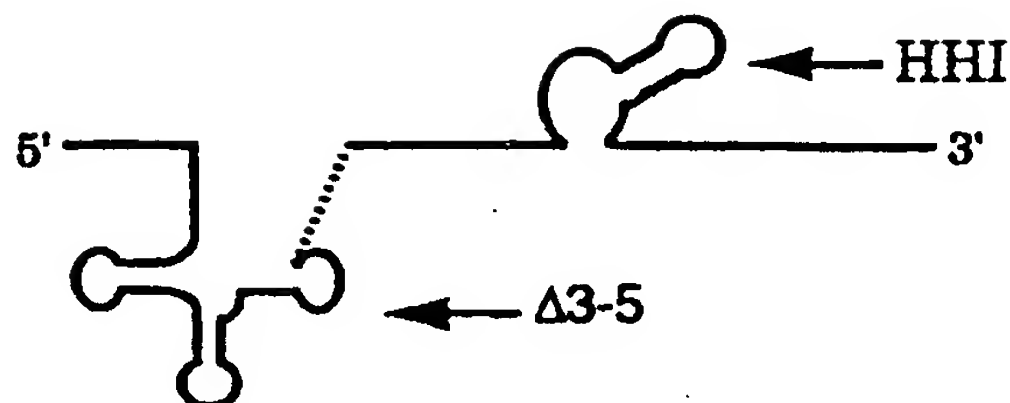


FIG. 34b.
S3

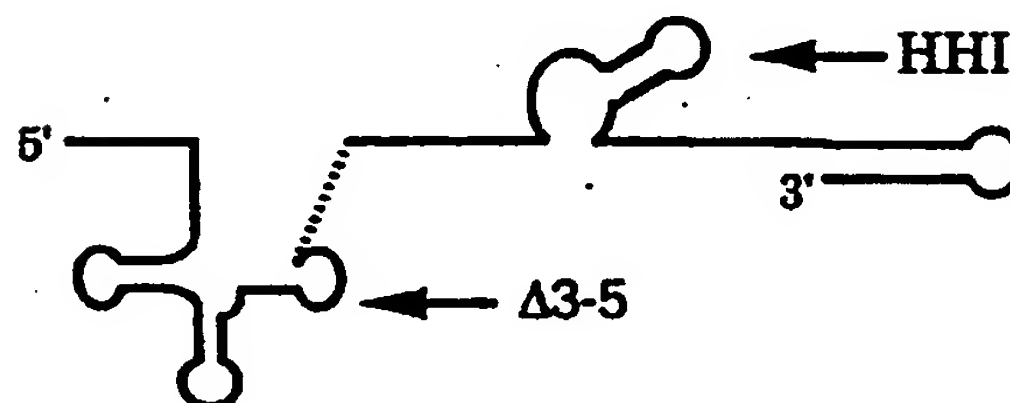


FIG. 34c.
S5

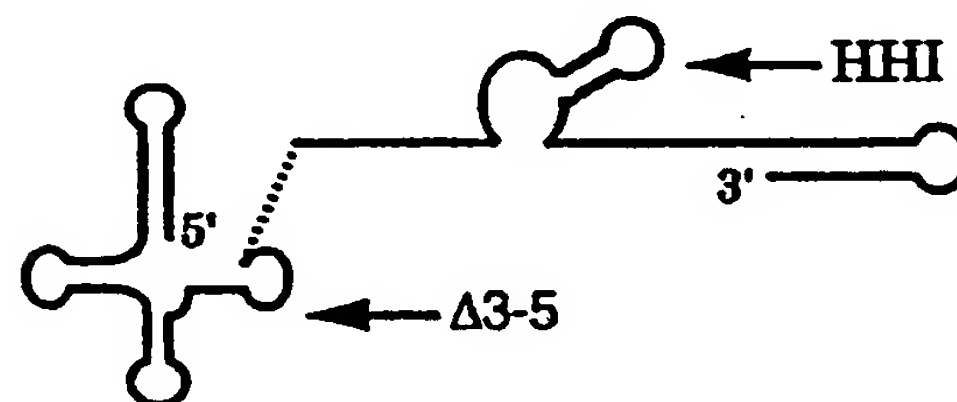


FIG. 34d.
S35

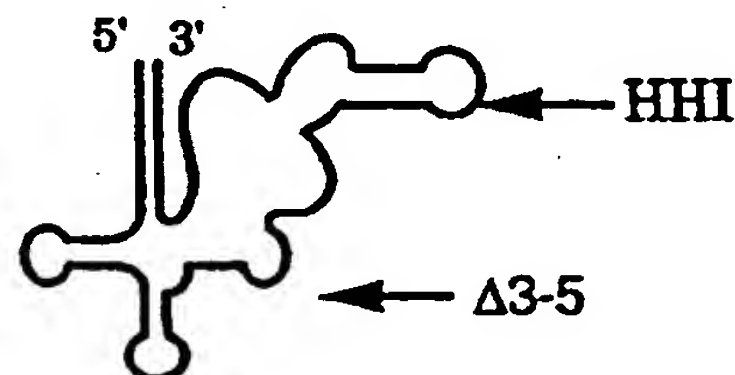
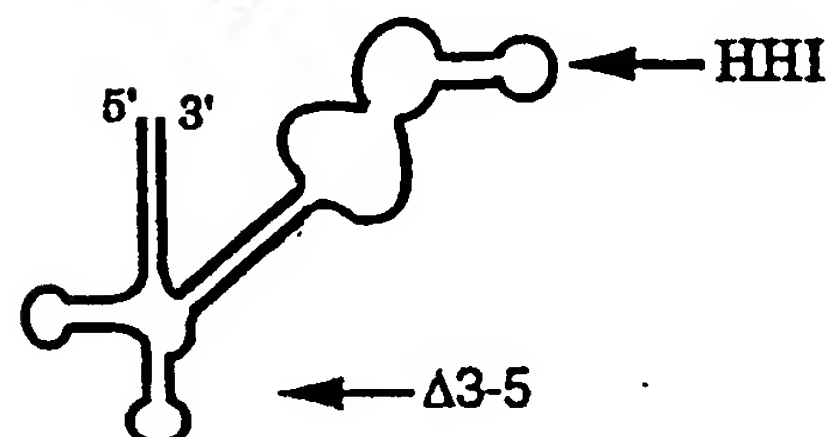


FIG. 34e.
S35Plus



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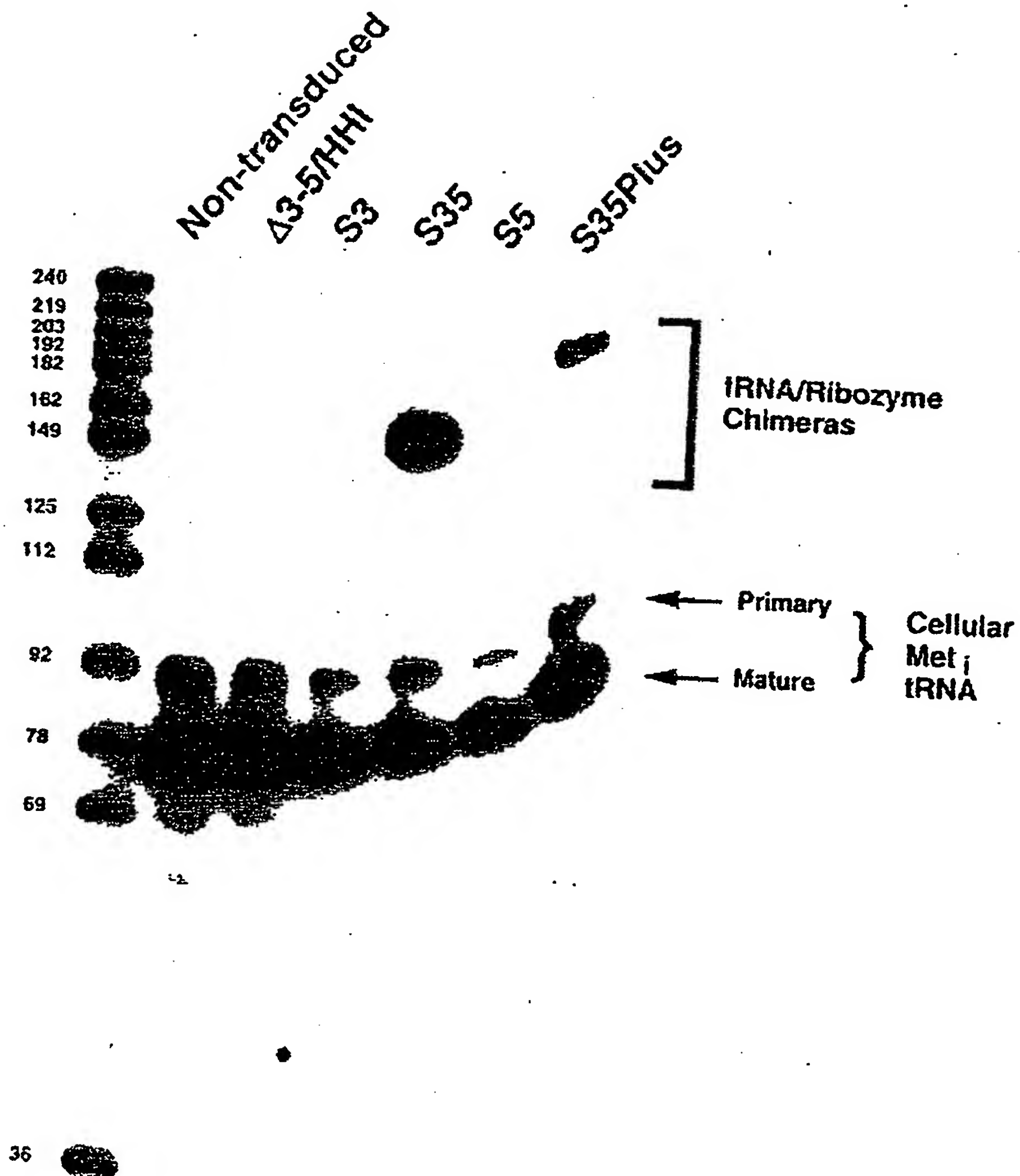


FIG. 35.

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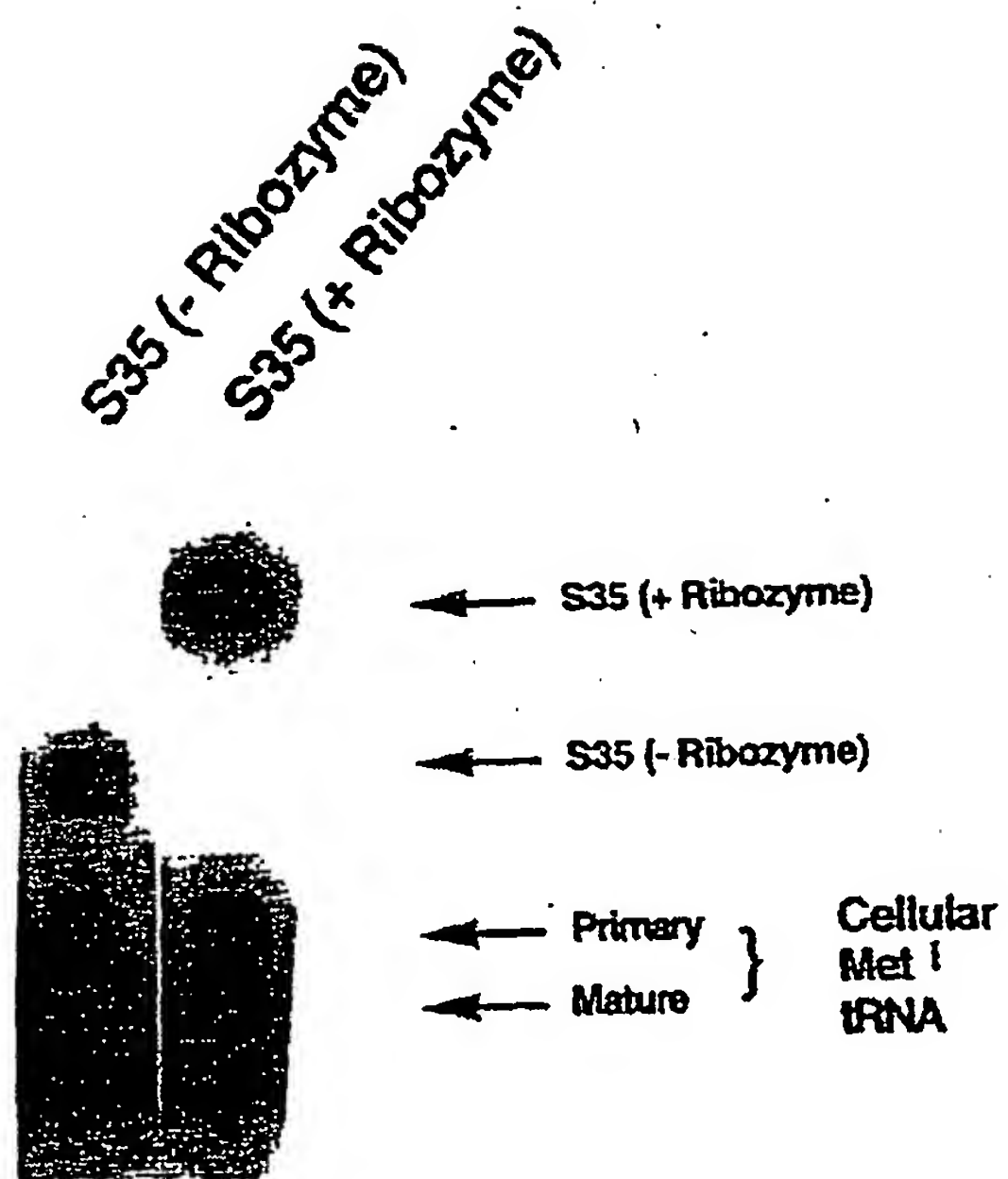


FIG. 36.

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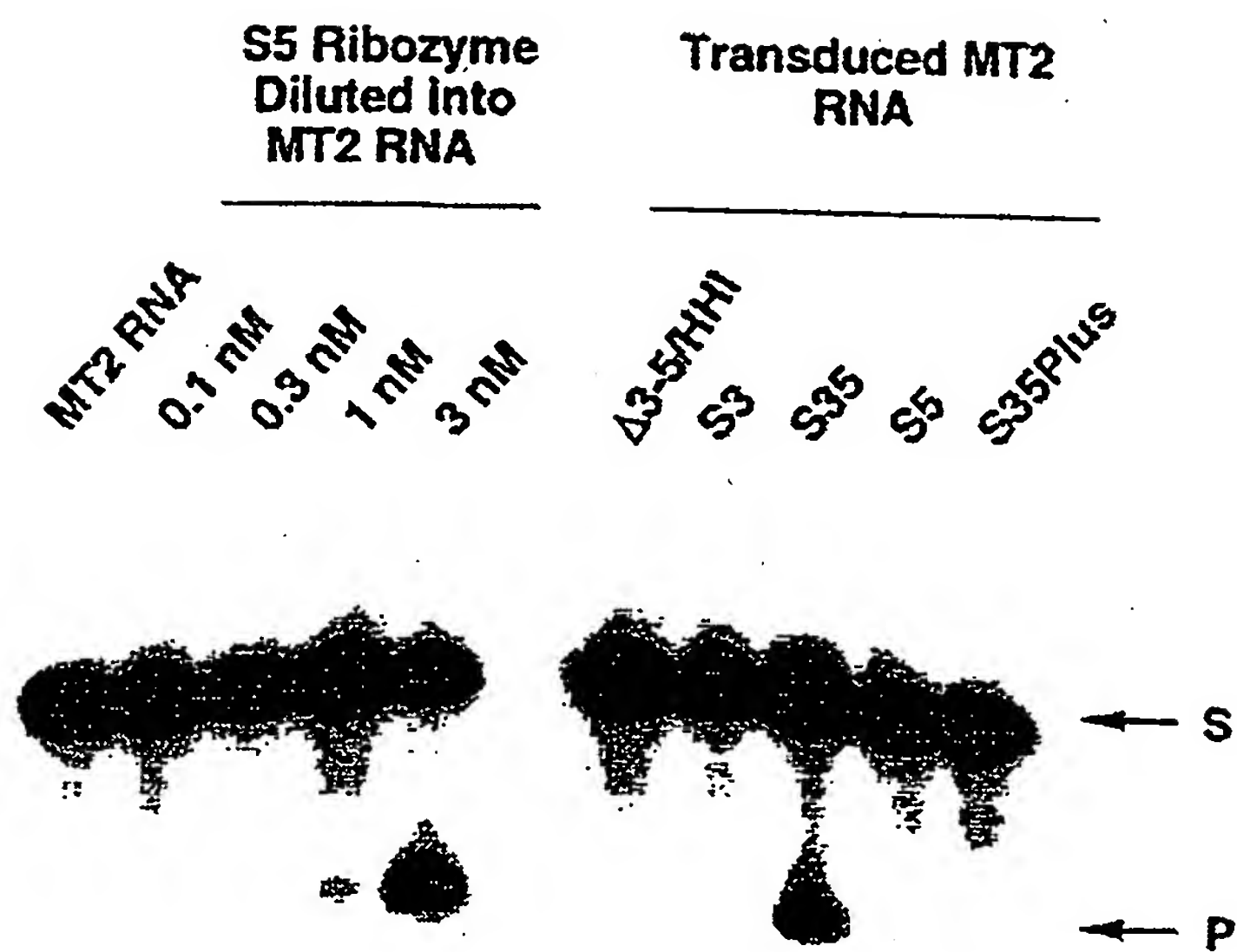


FIG. 37.

SUBSTITUTE SHEET (RULE 26)

NUC 37943

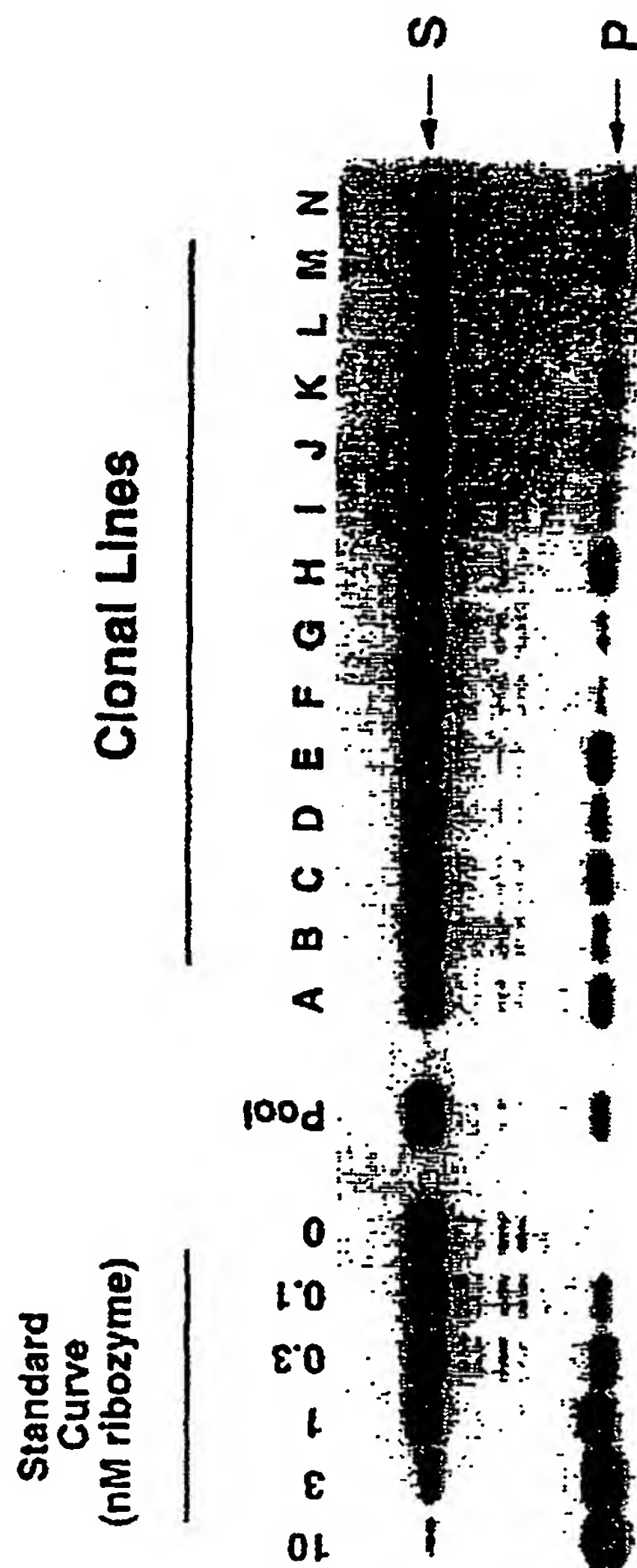
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FIG. 38.



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FIG. 39.



SUBSTITUTE SHEET (RULE 26)

NUC 37945

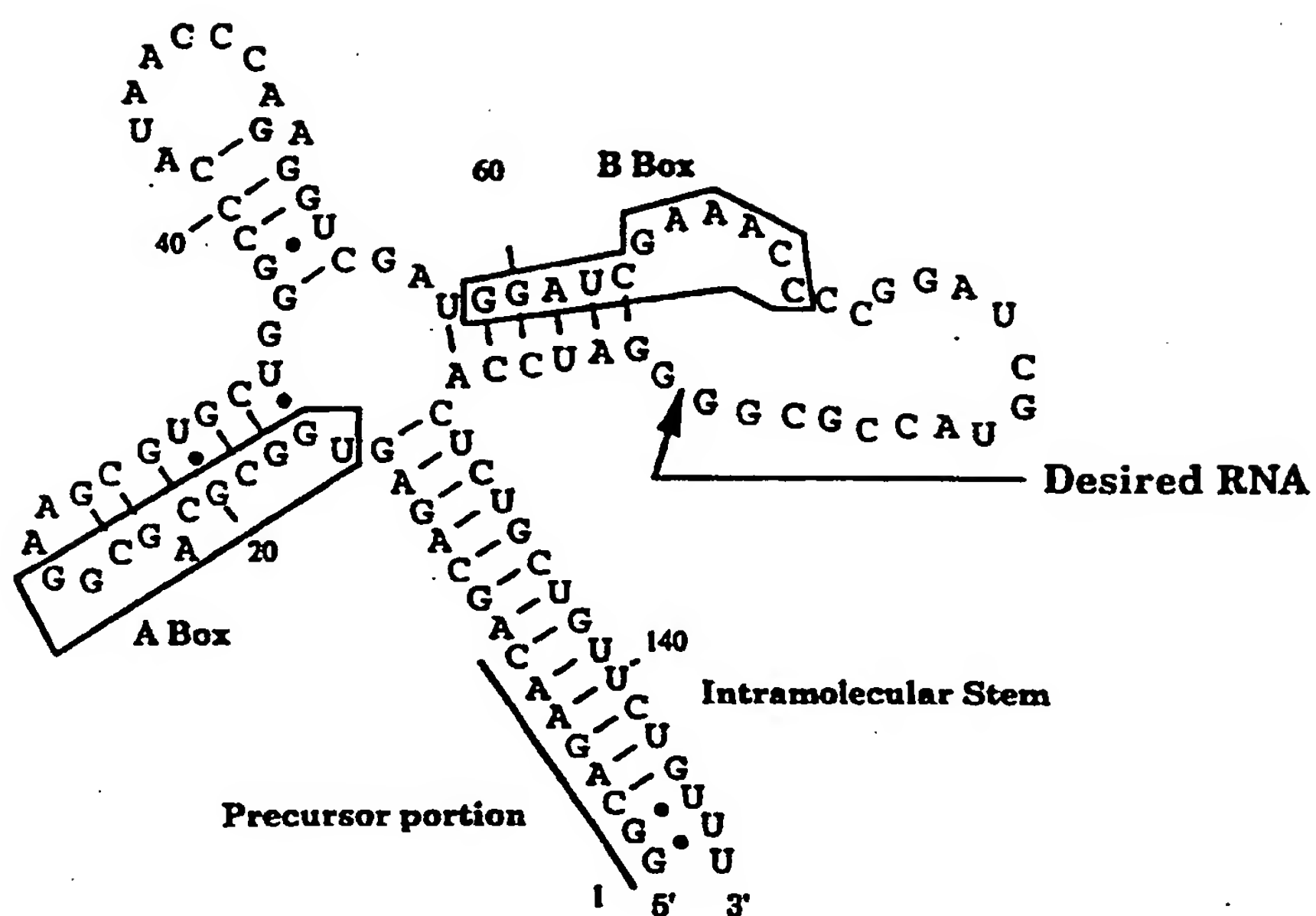


FIG. 40.

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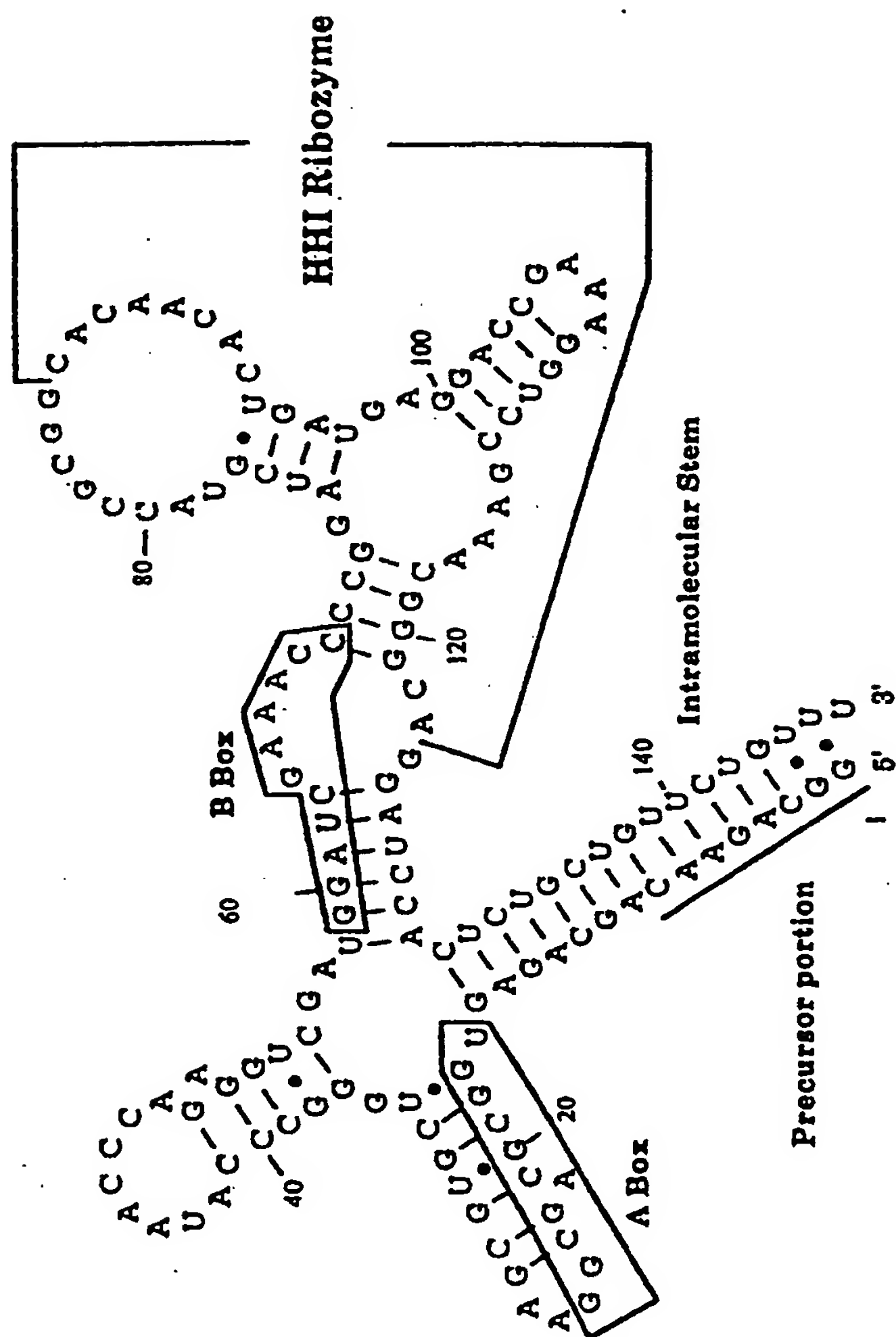


FIG. 41.

SUBSTITUTE SHEET (RULE 26)

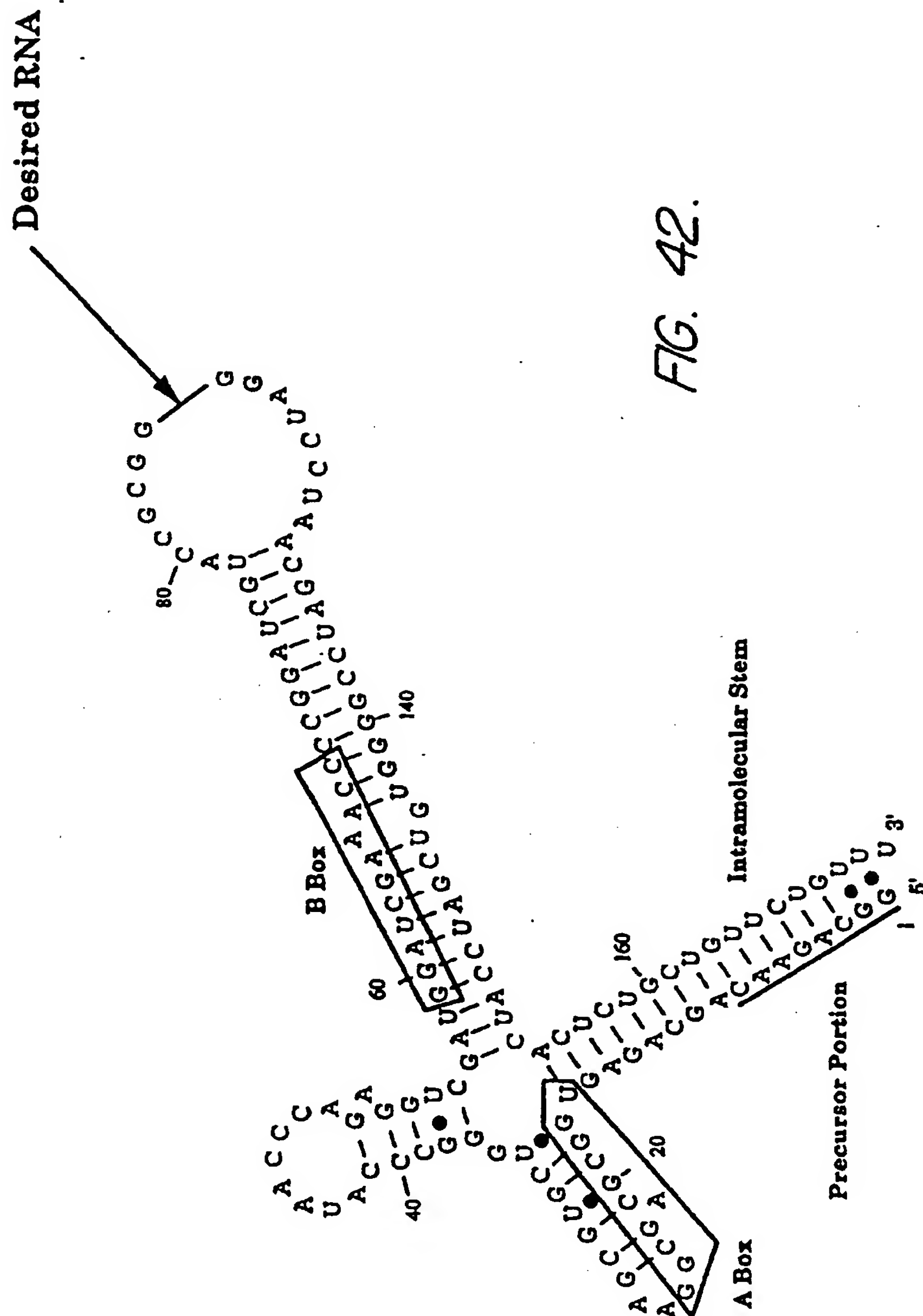


FIG. 42.

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NUC 37948

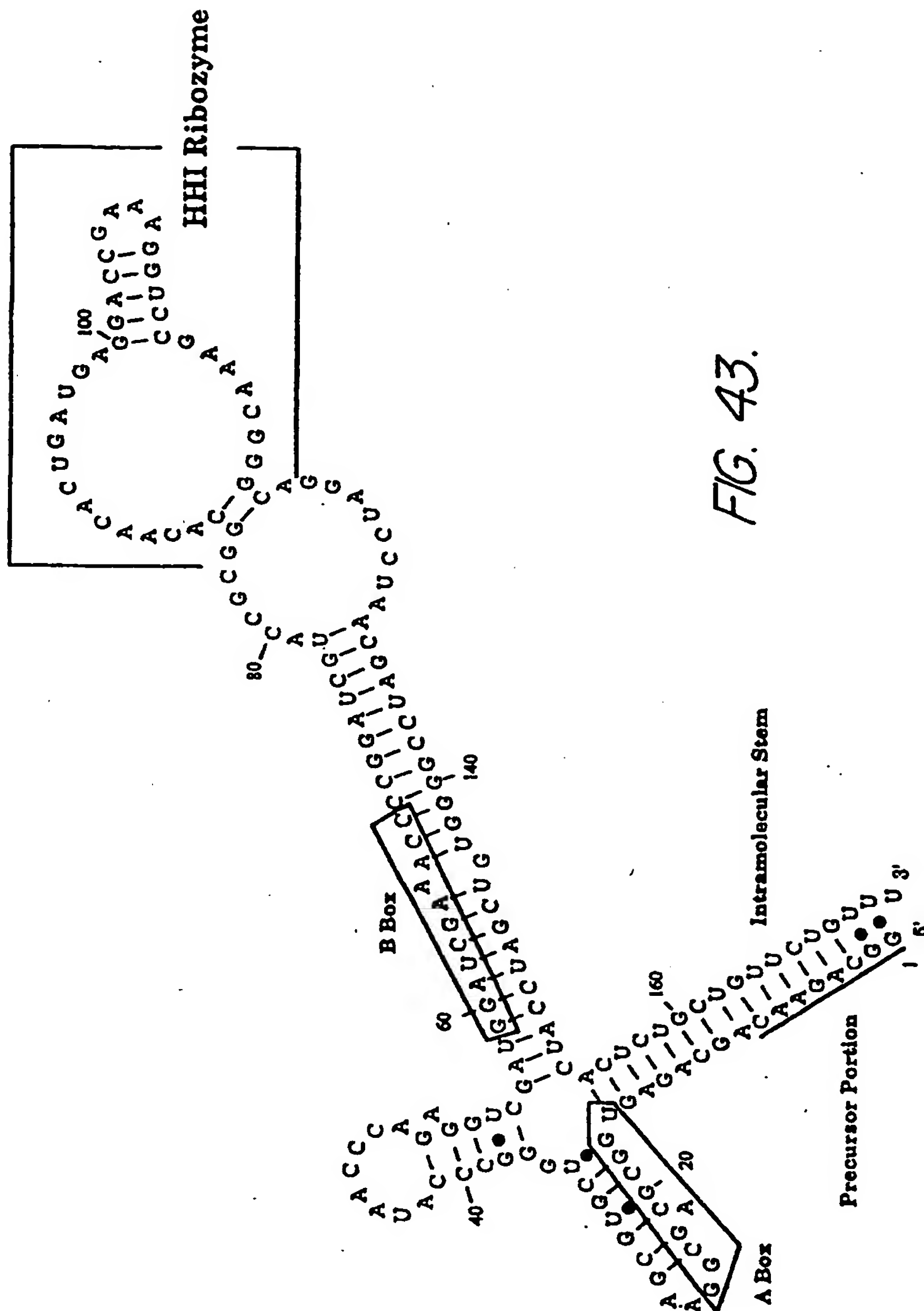


FIG. 43.

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FIG. 44.

S35 Sequence

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGUGGAU CCACUCUGCU 100
 GUUCUGUUU 109

FIG. 45.

HHIS35

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGCACAA CACUGAUGAG 100
GACCGAAAGG UCCGAAACGG GCAGGAUCCA CUCUGCUGUU CUGUUU 146

Underlined bases indicate the HHI ribozyme sequence

FIG. 46.

S35 Plus Sequence

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGGGAUC CUAACGAUCC 100
 GGGGUGUCGA UCCAUCACUC UGCUGUUCUG UU U 133

FIG. 47.

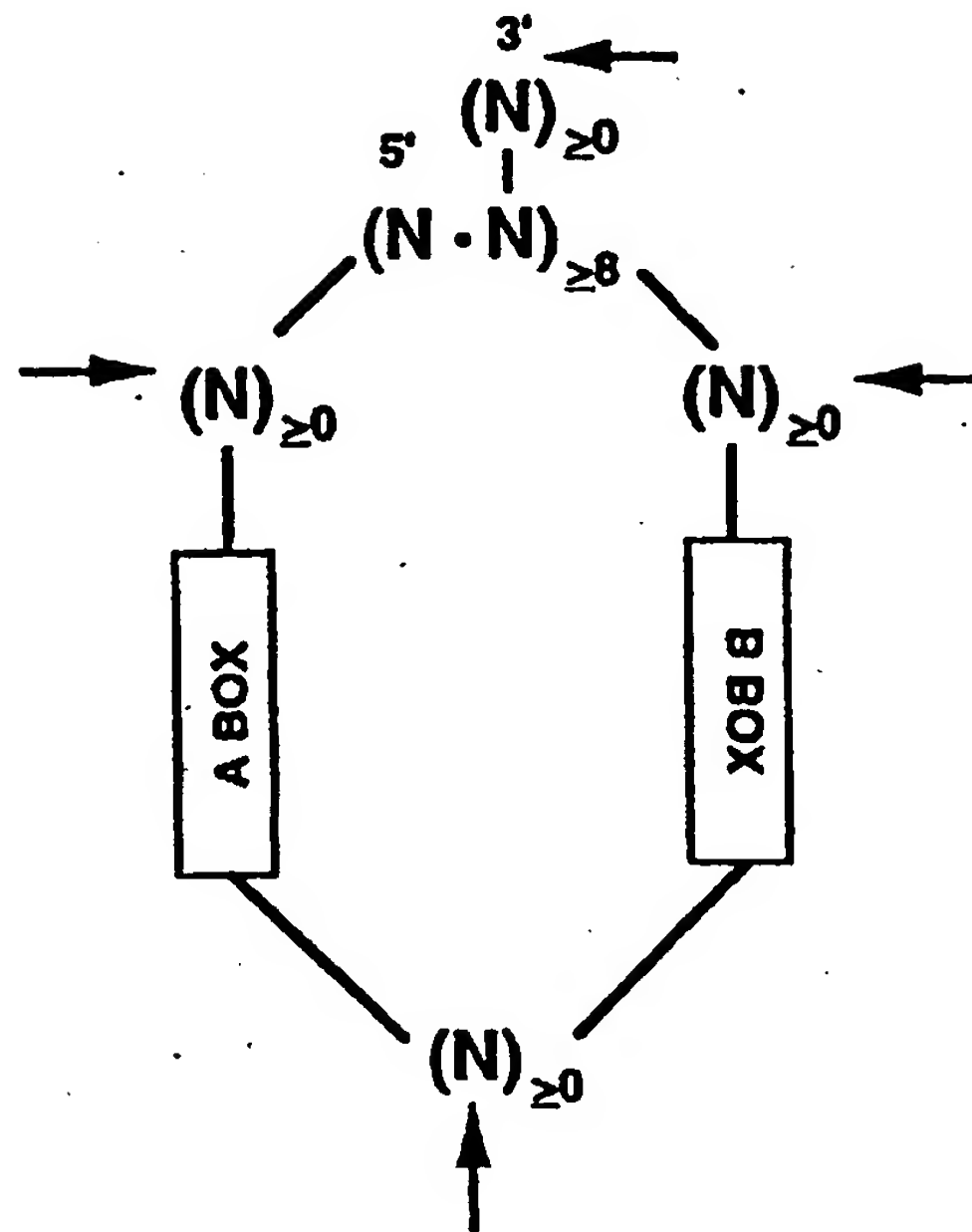
HHIS35 Plus

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGCACAA CACUGAUGAG 100
GACCGAAAGG UCCGAAACGG GCAGGAUCCU AACGAUCCGG GGUGUCGAUC 150
 CAUCACUCUG CUGUUCUGUU U 171

Underlined bases indicate the HHI ribozyme sequence
 SUBSTITUTE SHEET (RULE 26)

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FIG. 48.



A BOX = URG C N N A G Y G G

B BOX = G G U U C G A N U C C

This is based on Geiduschek & Tocchini-Valentini, (1988) *Annu. Review Biochem.* 57, 873-914. However this consensus sequence is not meant to be limiting

N = A, U, G, or C

R = Purine

Y = Pyrimidine

• = Indicates base-pairing

— = Indicates covalent linkage

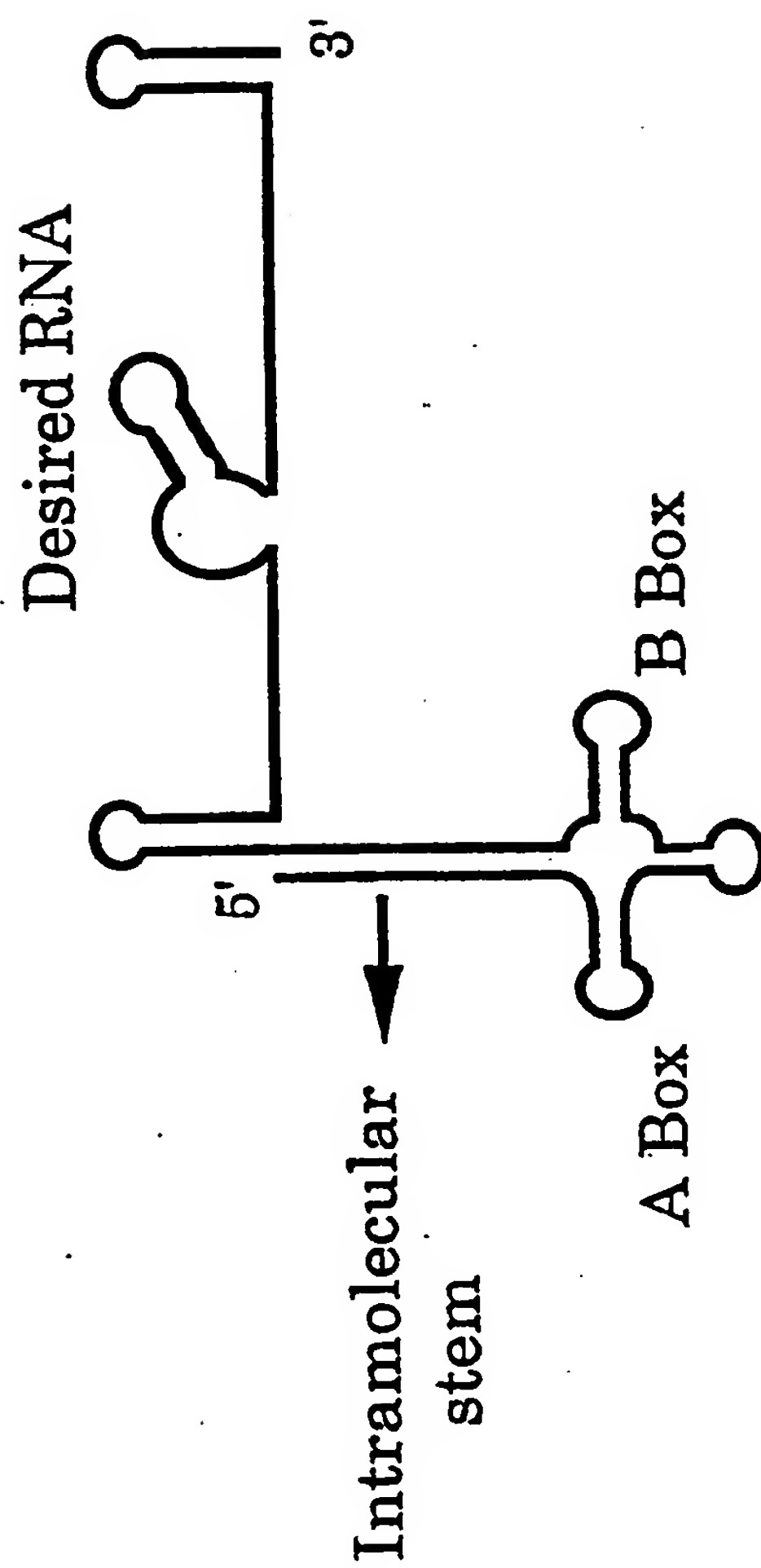
➔ = Indicates sites at which desired RNAs can be cloned

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FIG. 49.



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FIG. 52a.

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A: TRZ-A

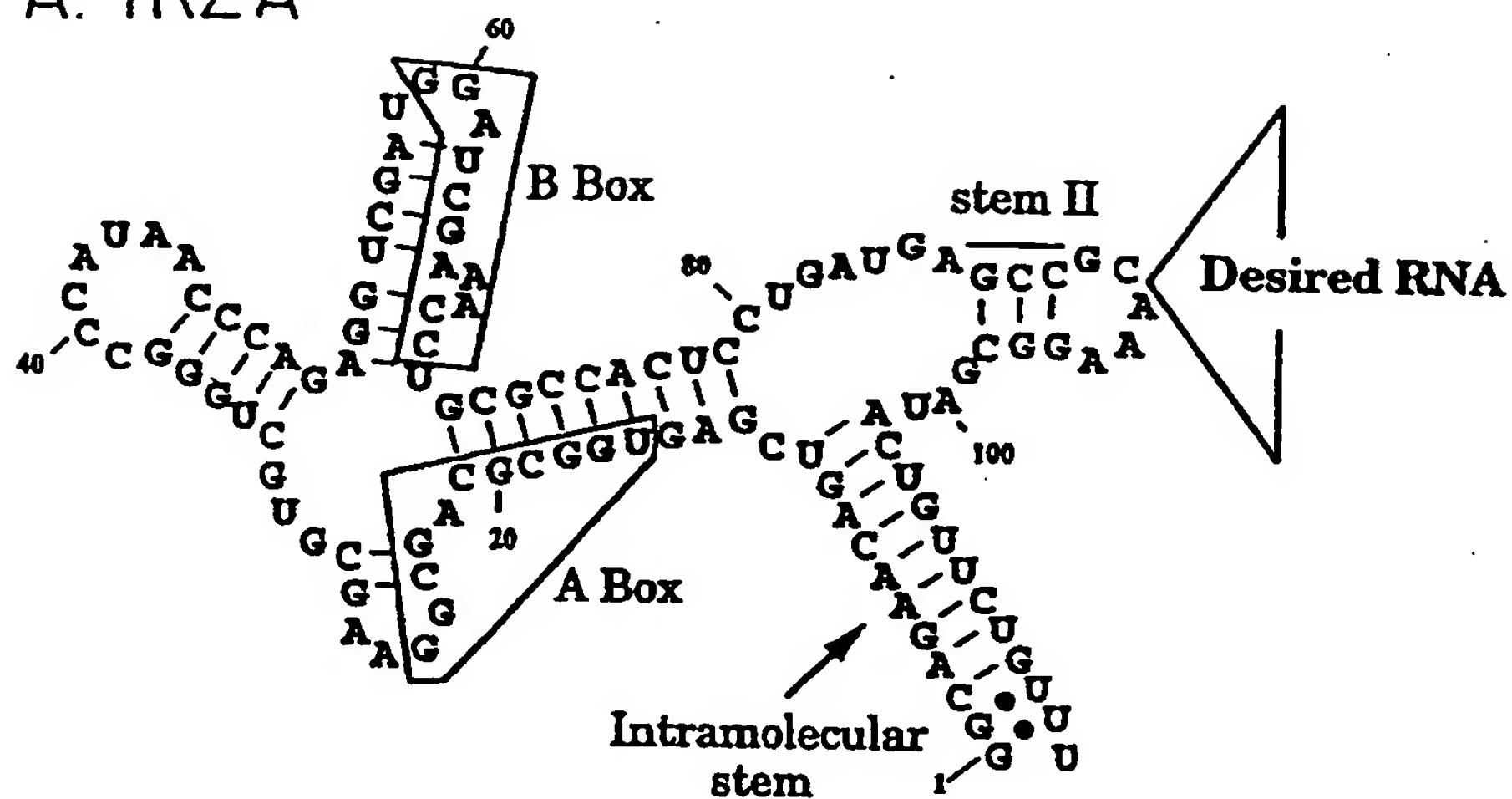
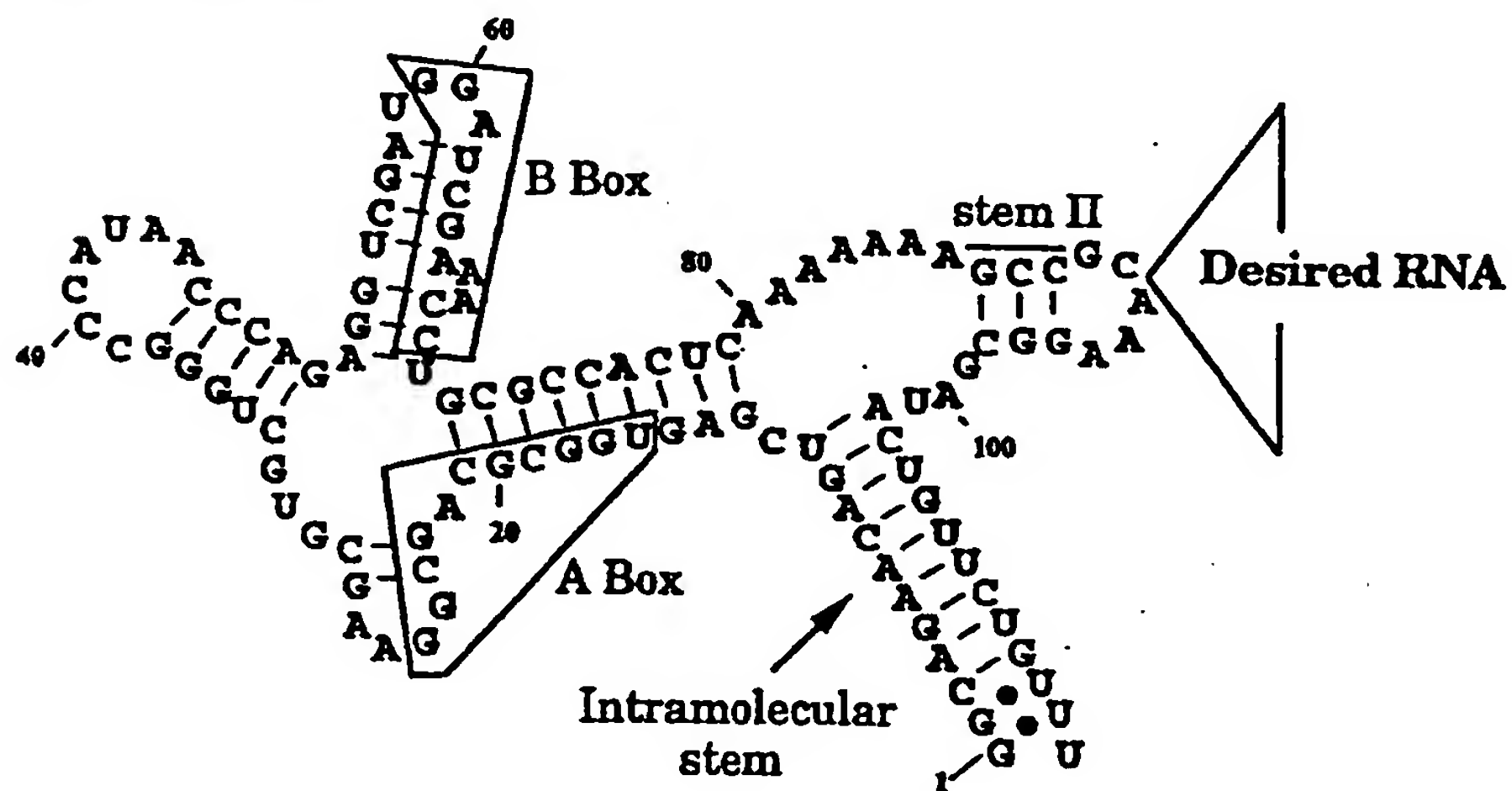


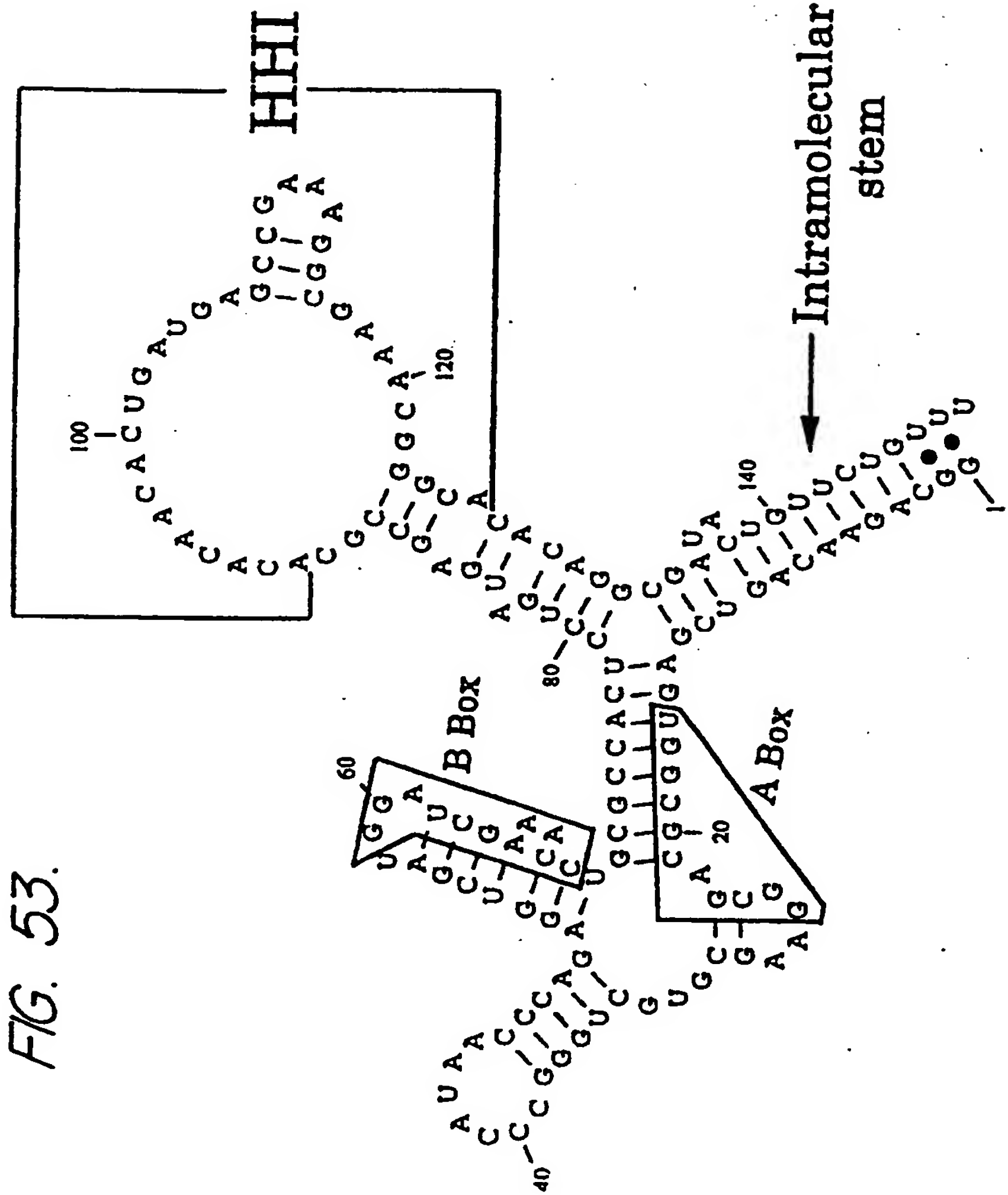
FIG. 52b.

B: TRZ-B



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NUC 37955



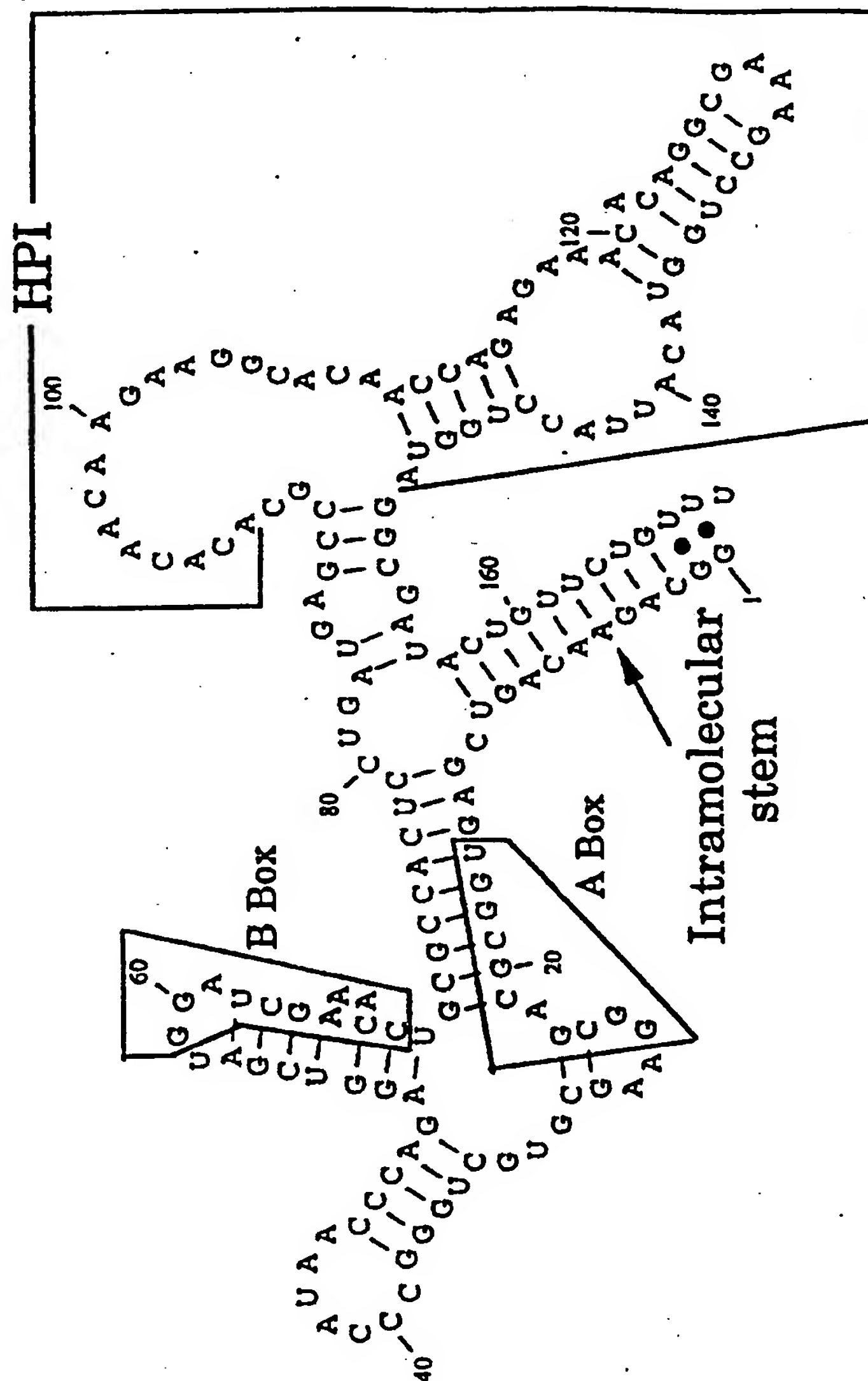


FIG. 54.

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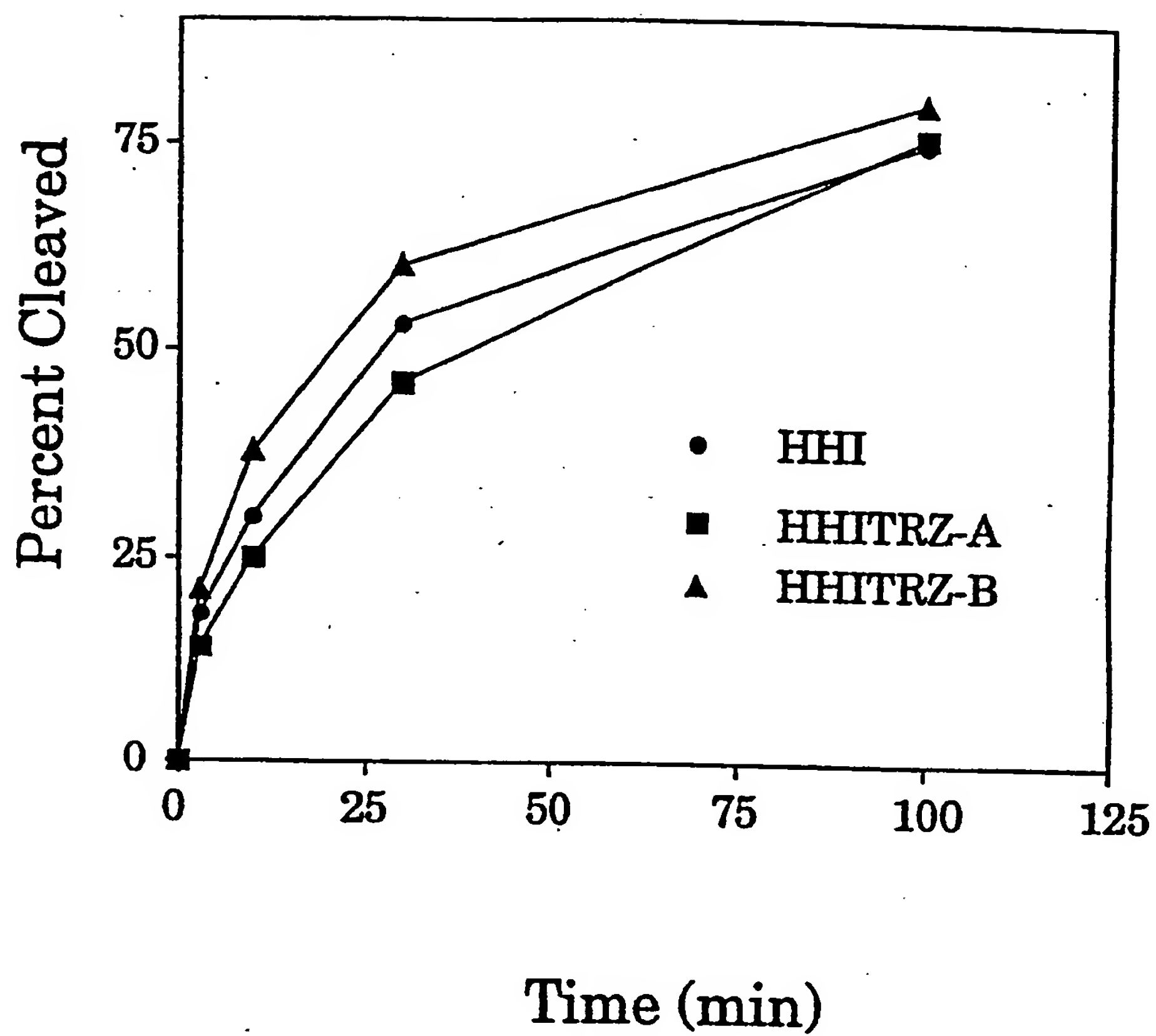


FIG. 55.

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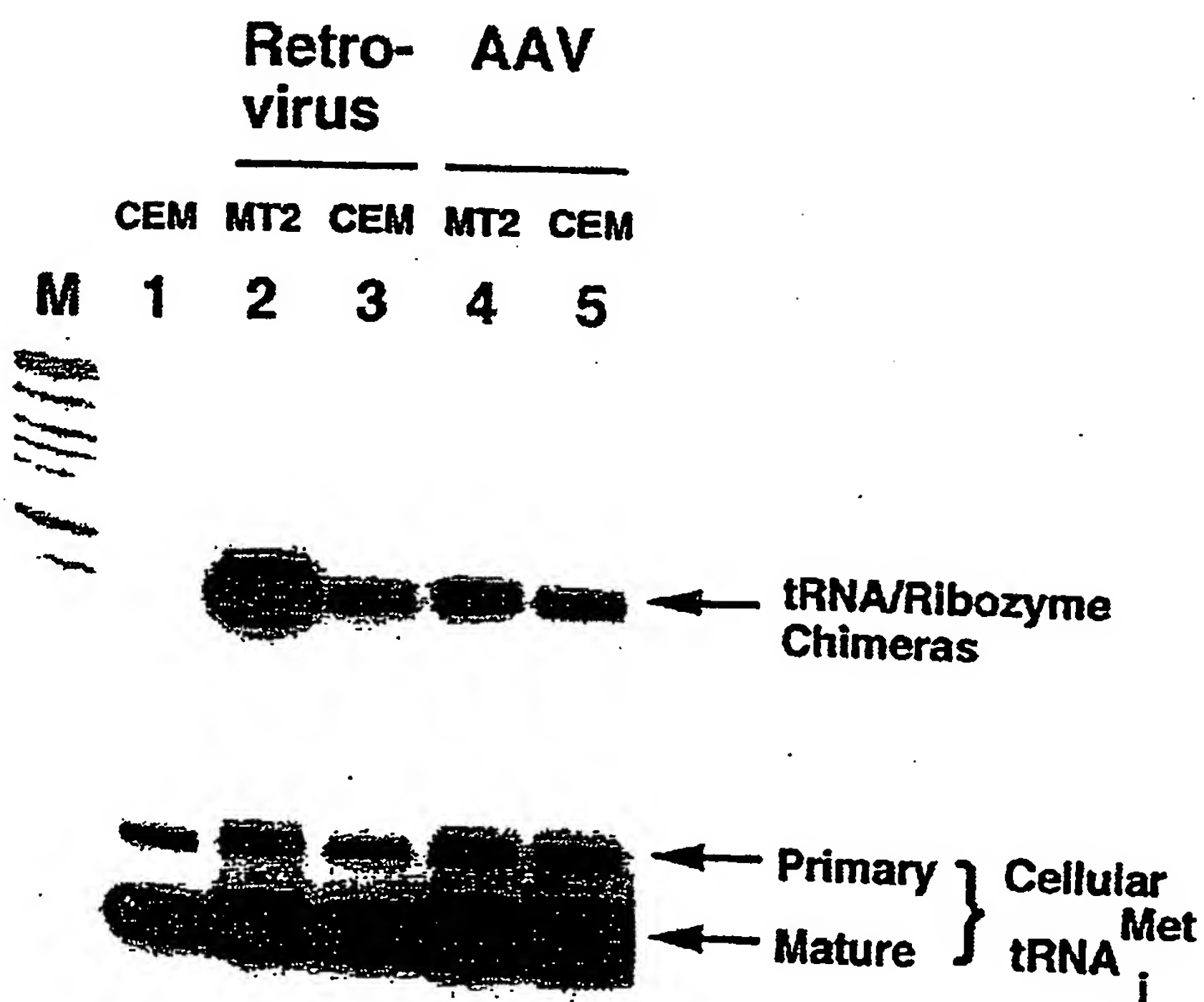


FIG. 56.

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FIG. 57a.

AAV Vector

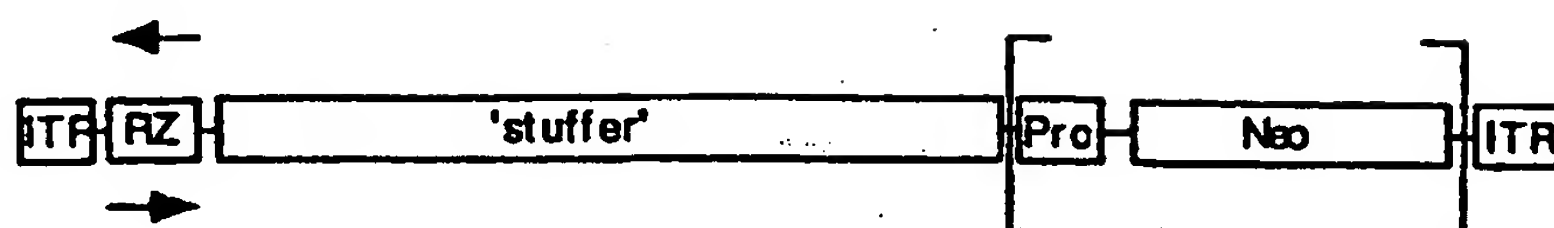
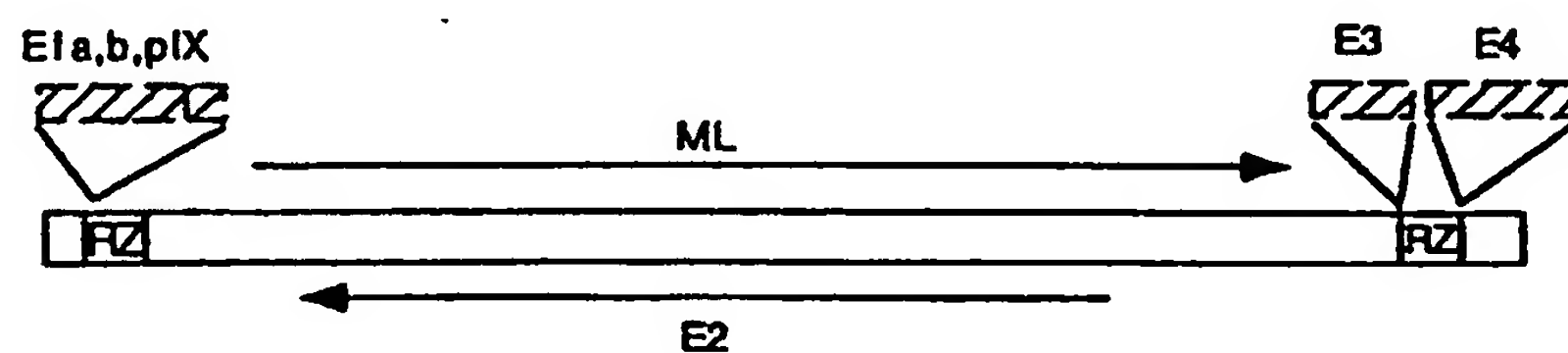


FIG. 57b.

Adenovirus Vector

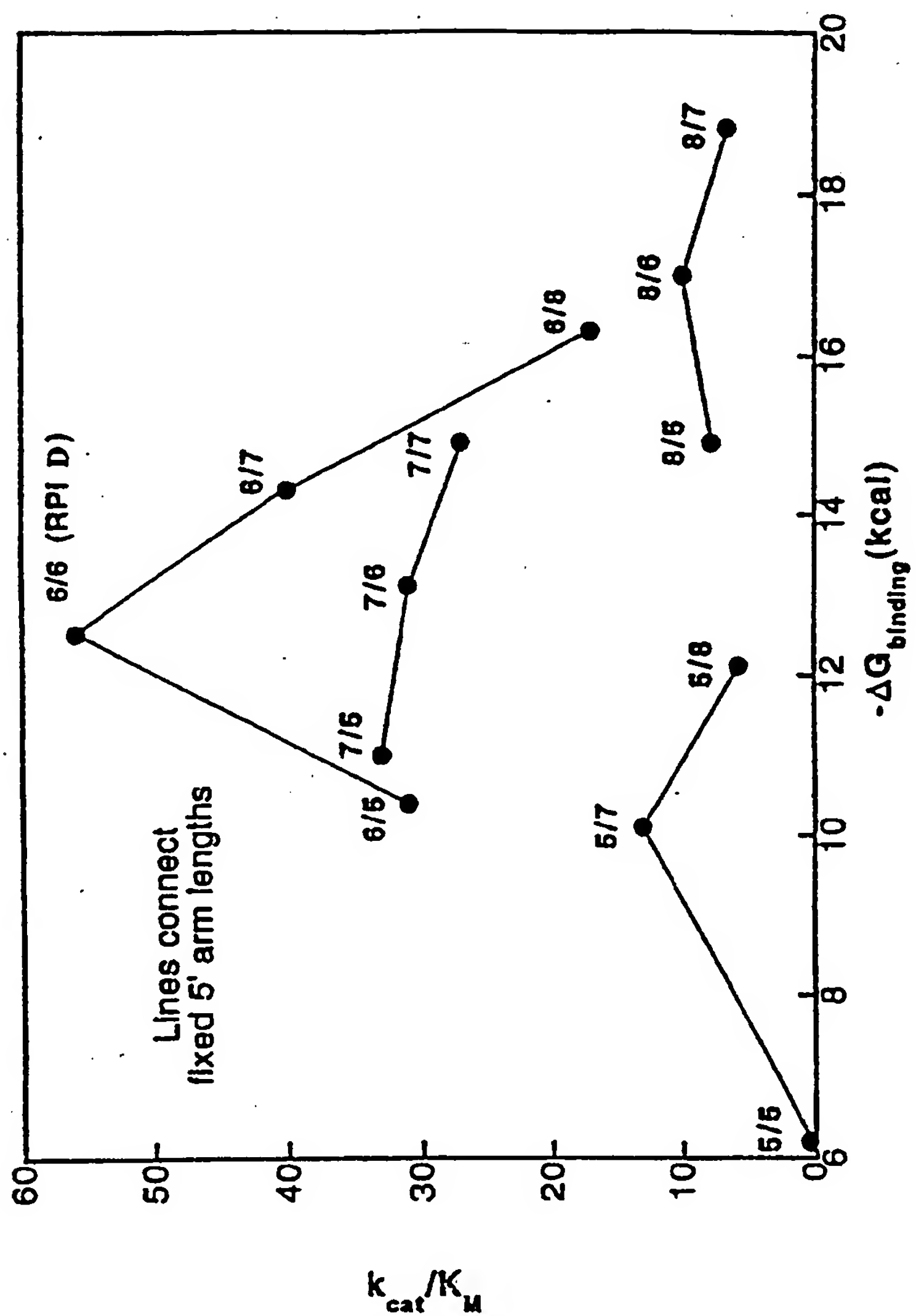


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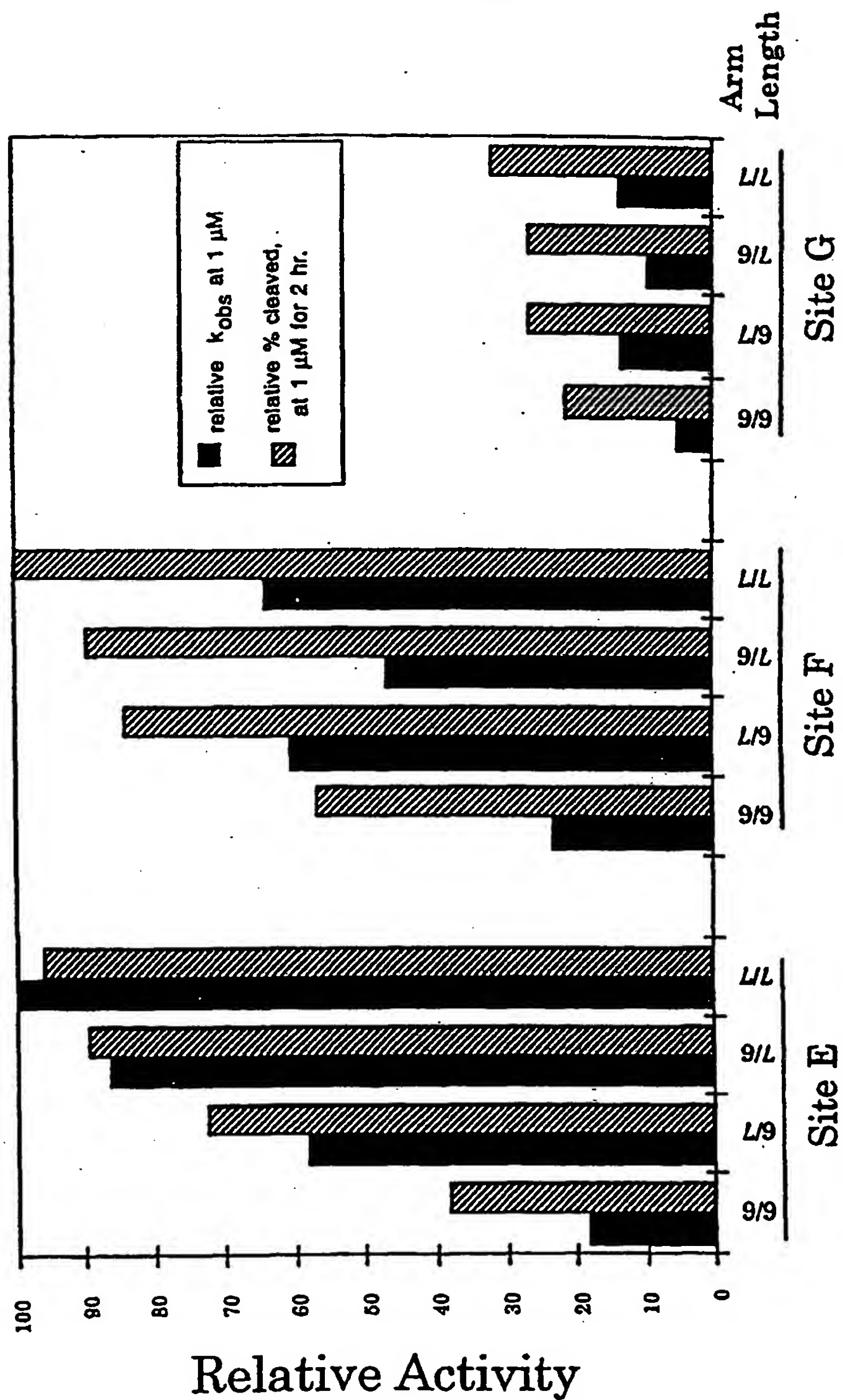
FIG. 58.



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Ribozyme

FIG. 59.

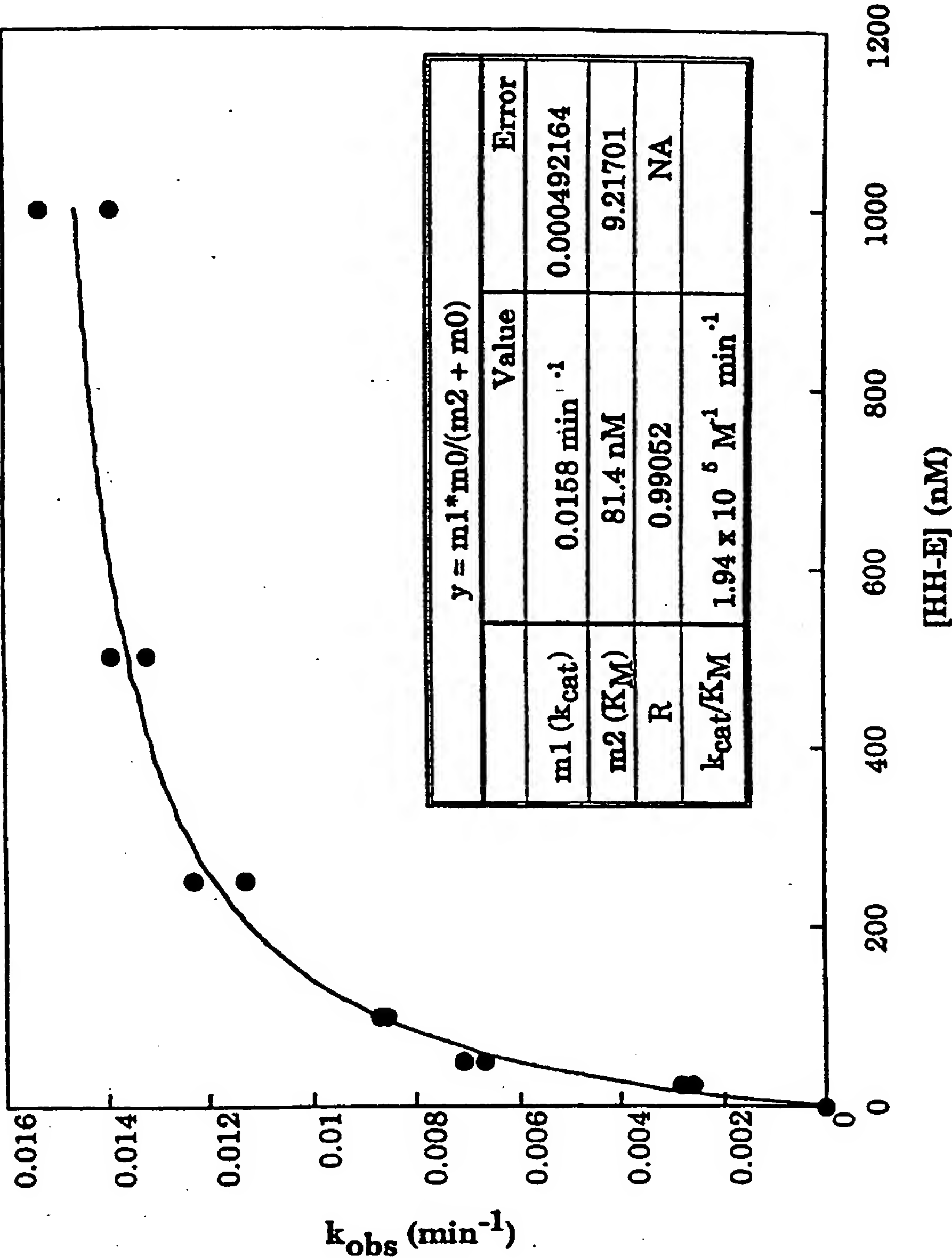
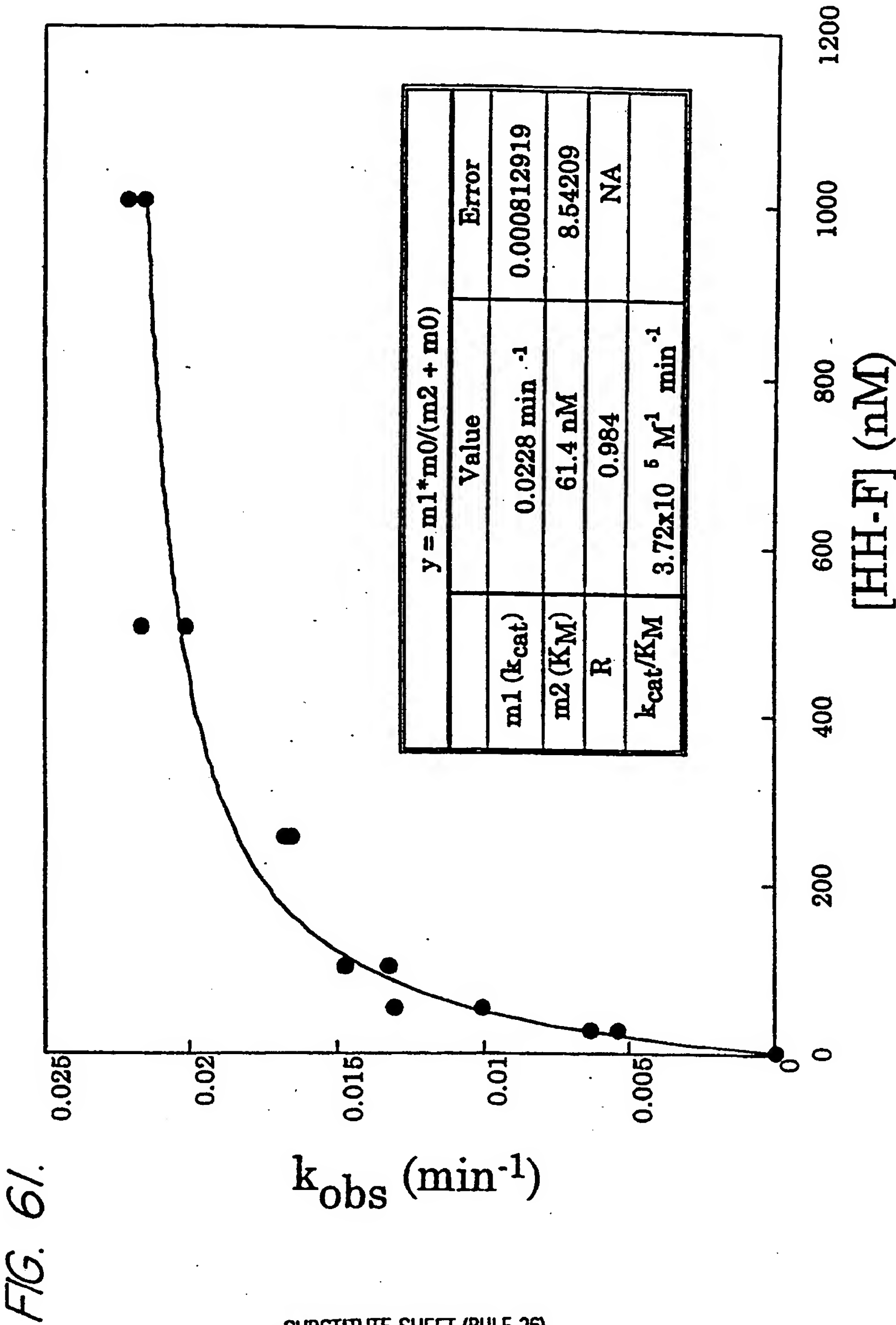


FIG. 60.

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SUBSTITUTE SHEET (RULE 26)

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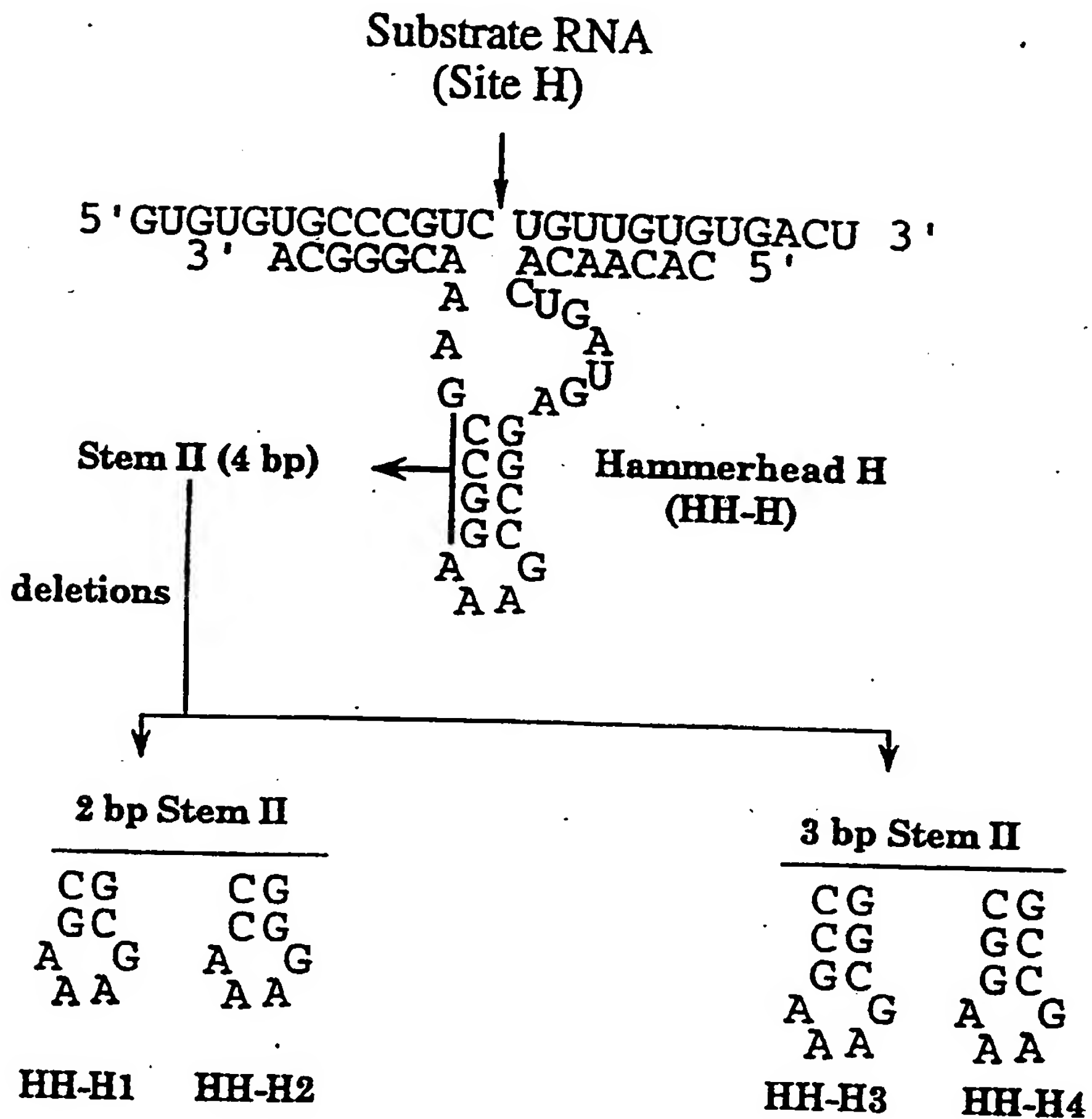


FIG. 62.

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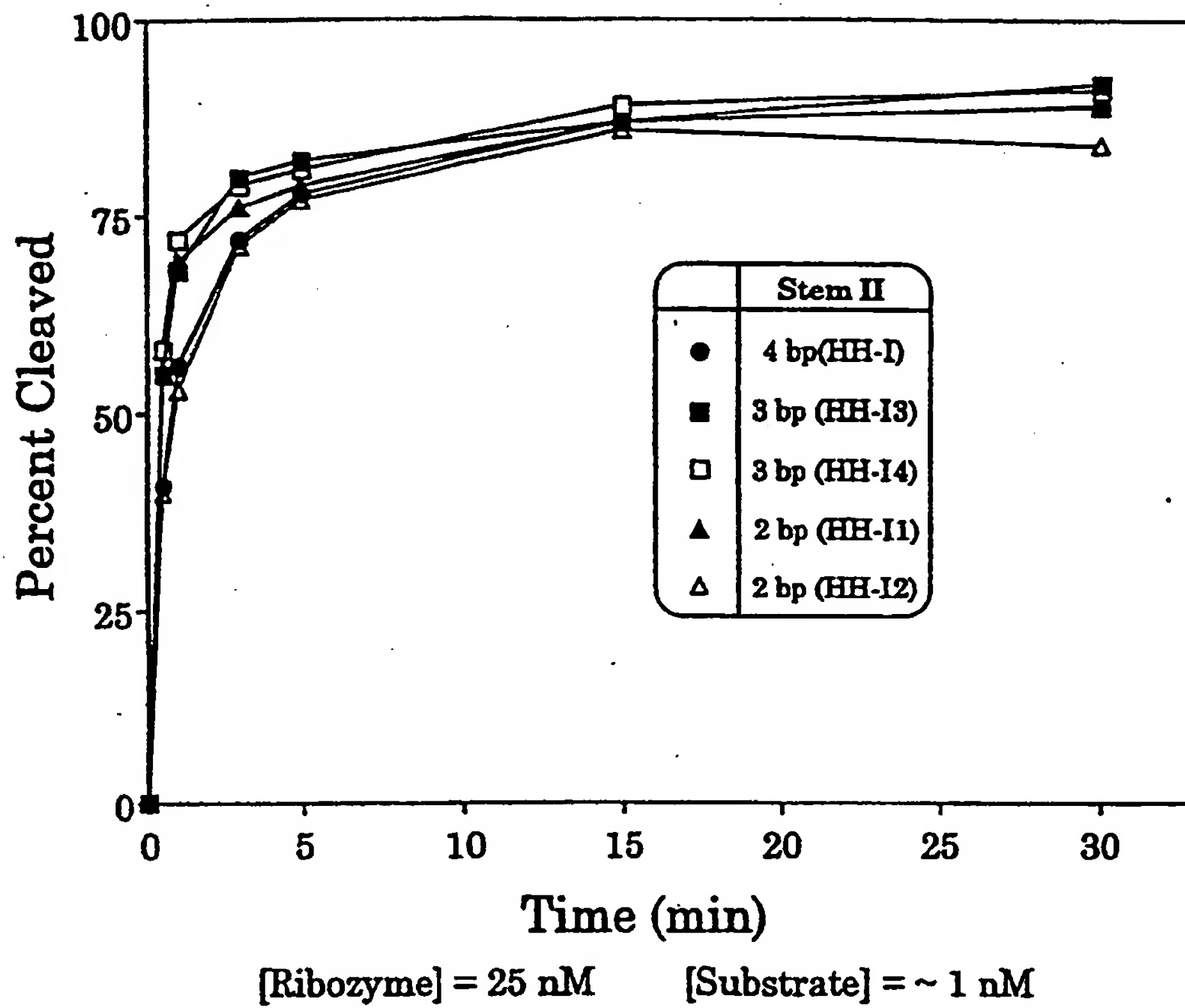


FIG. 63.

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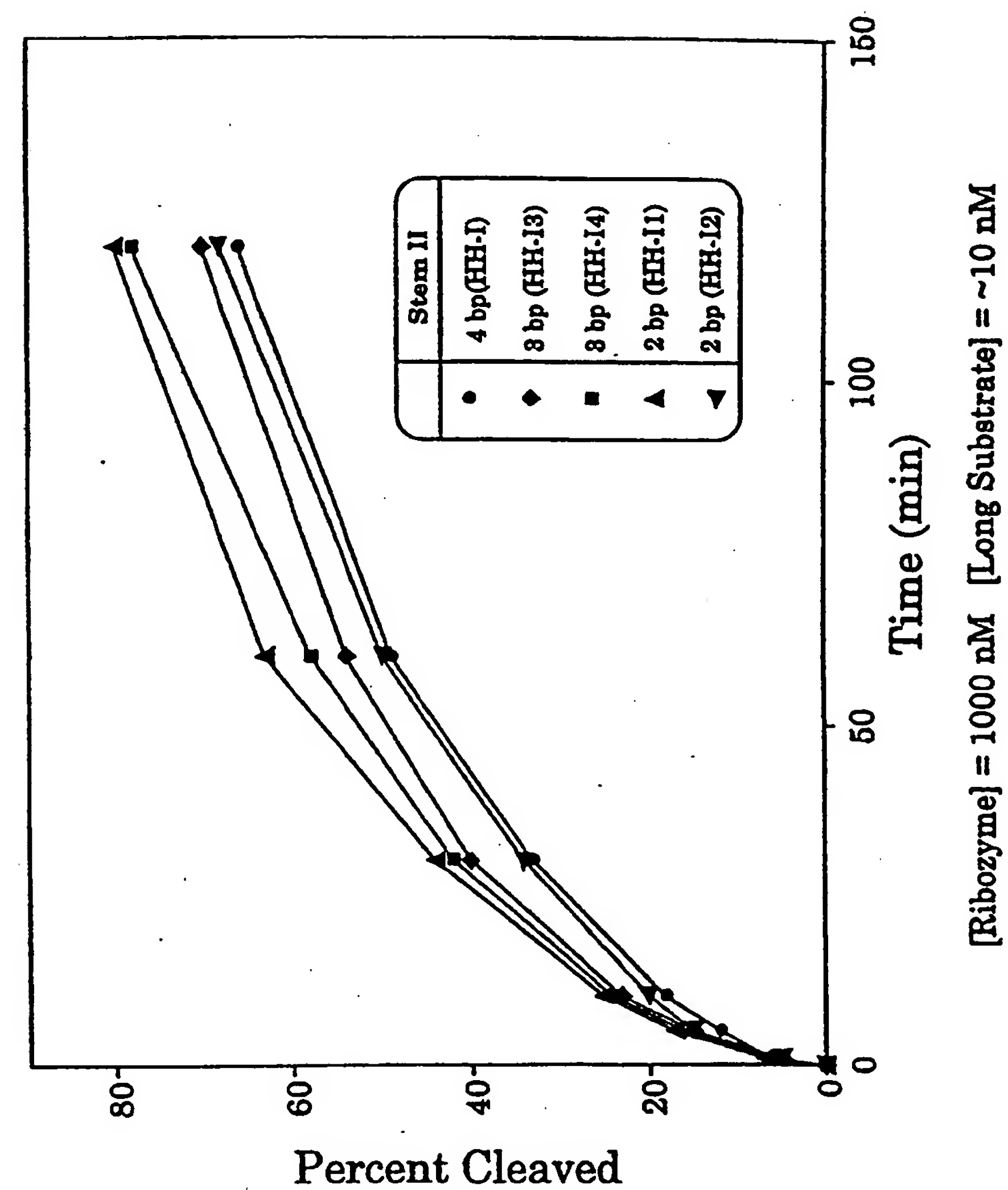


FIG. 64.

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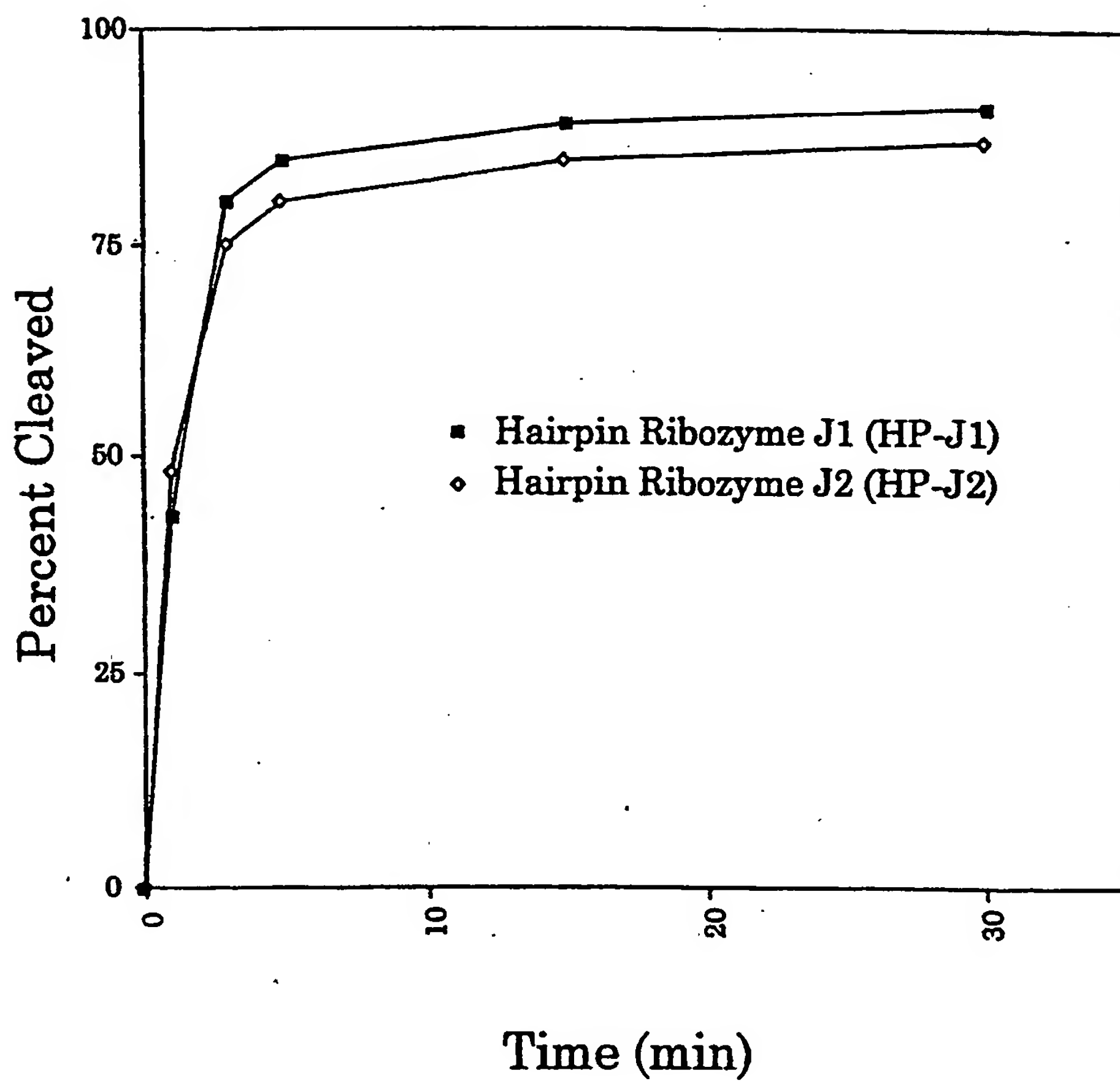


FIG. 66.

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FIG. 67b.

Substrate RNA

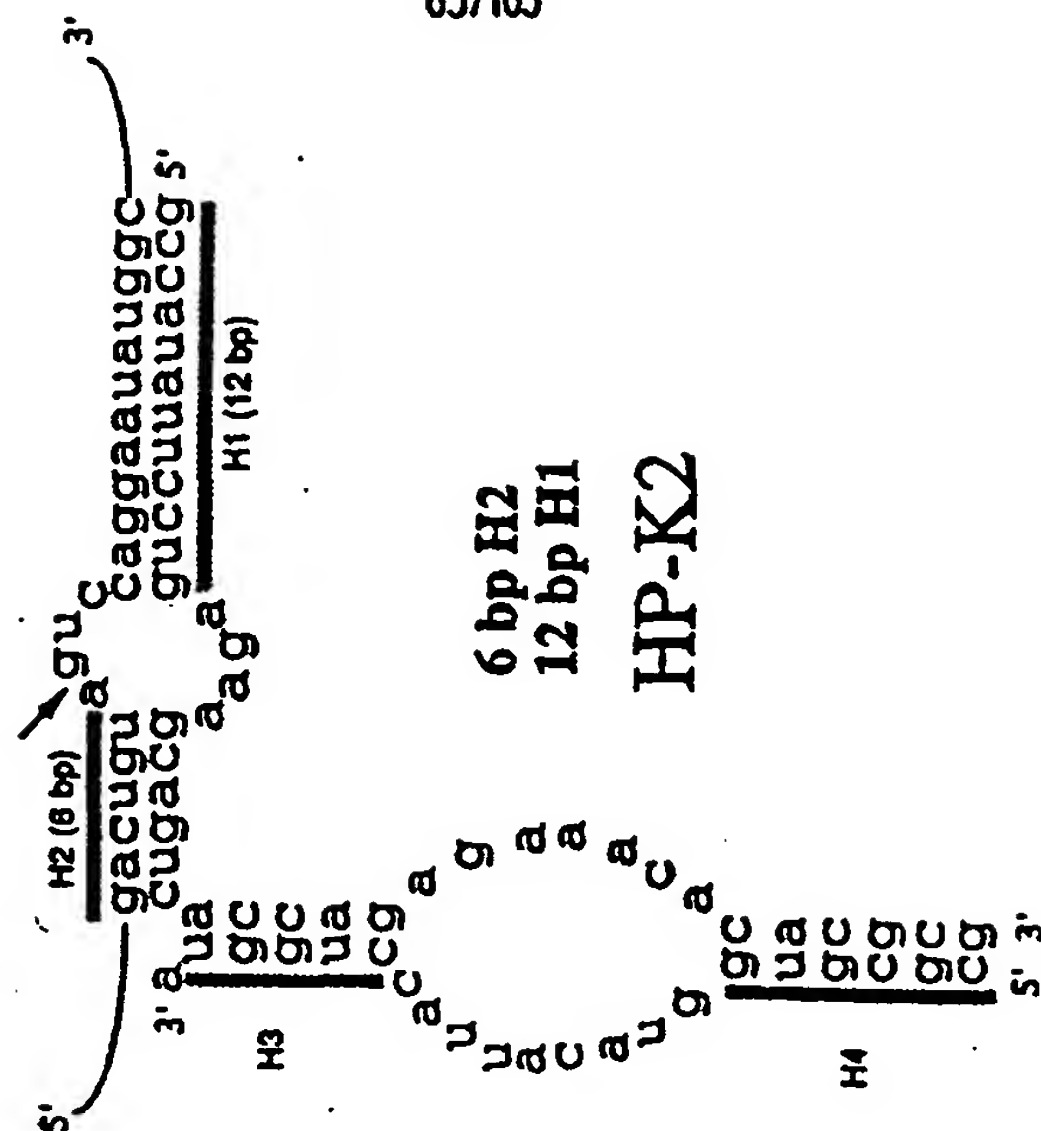
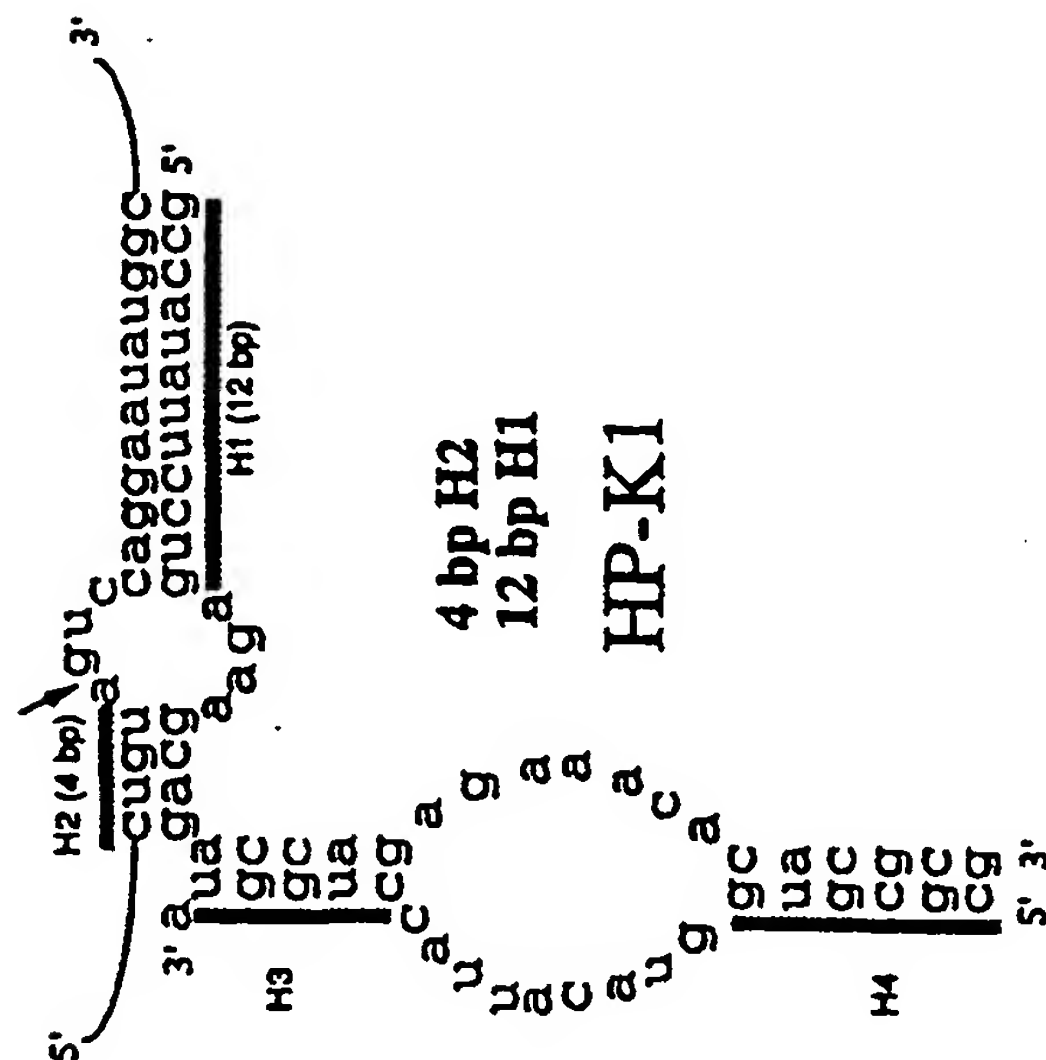


FIG. 67a.

Substrate RNA



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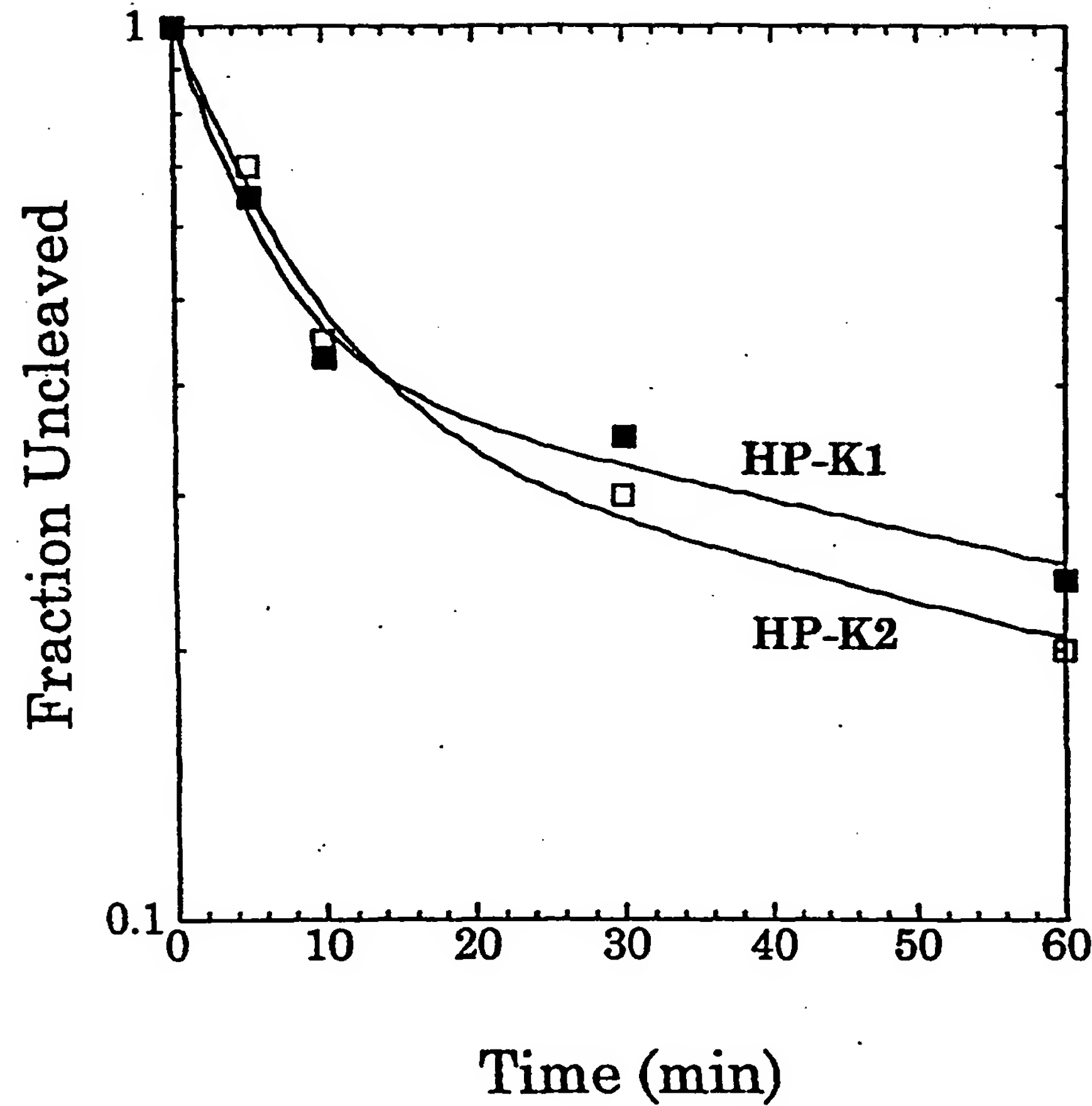


FIG. 68.

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FIG. 69b.

Substrate RNA

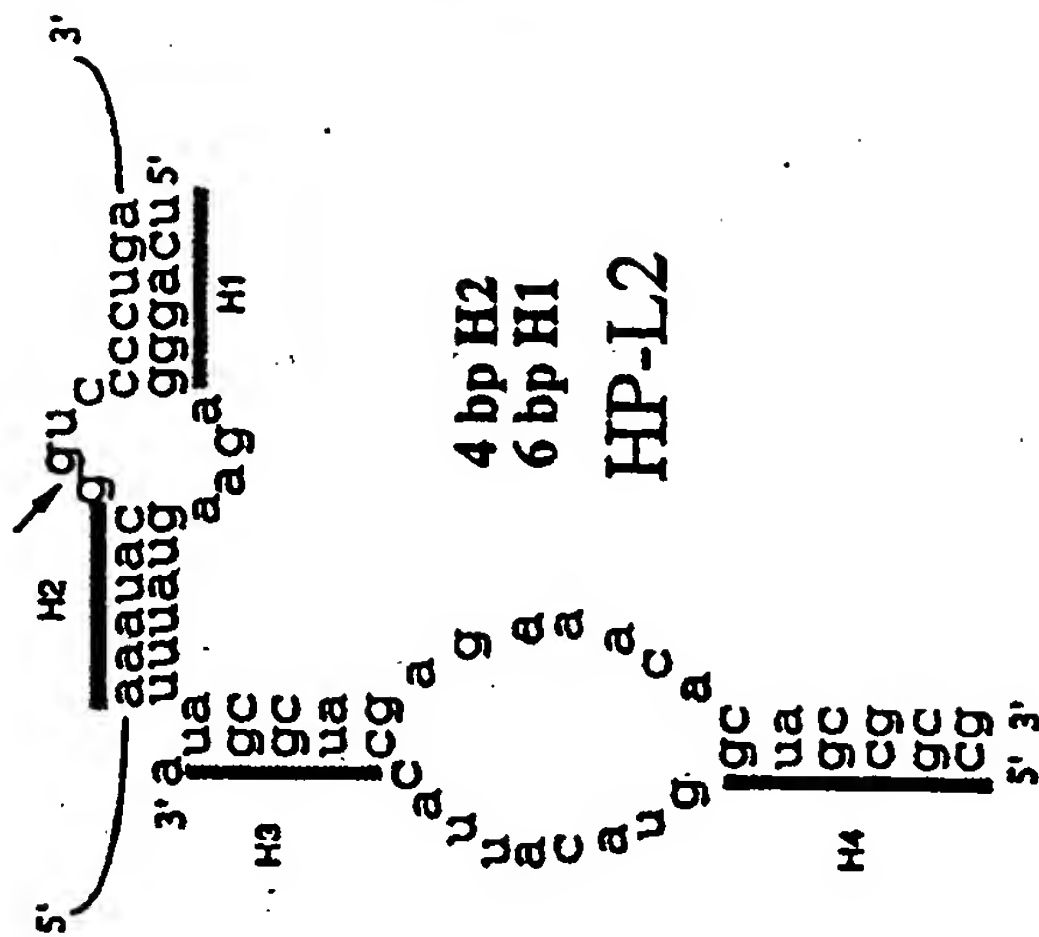
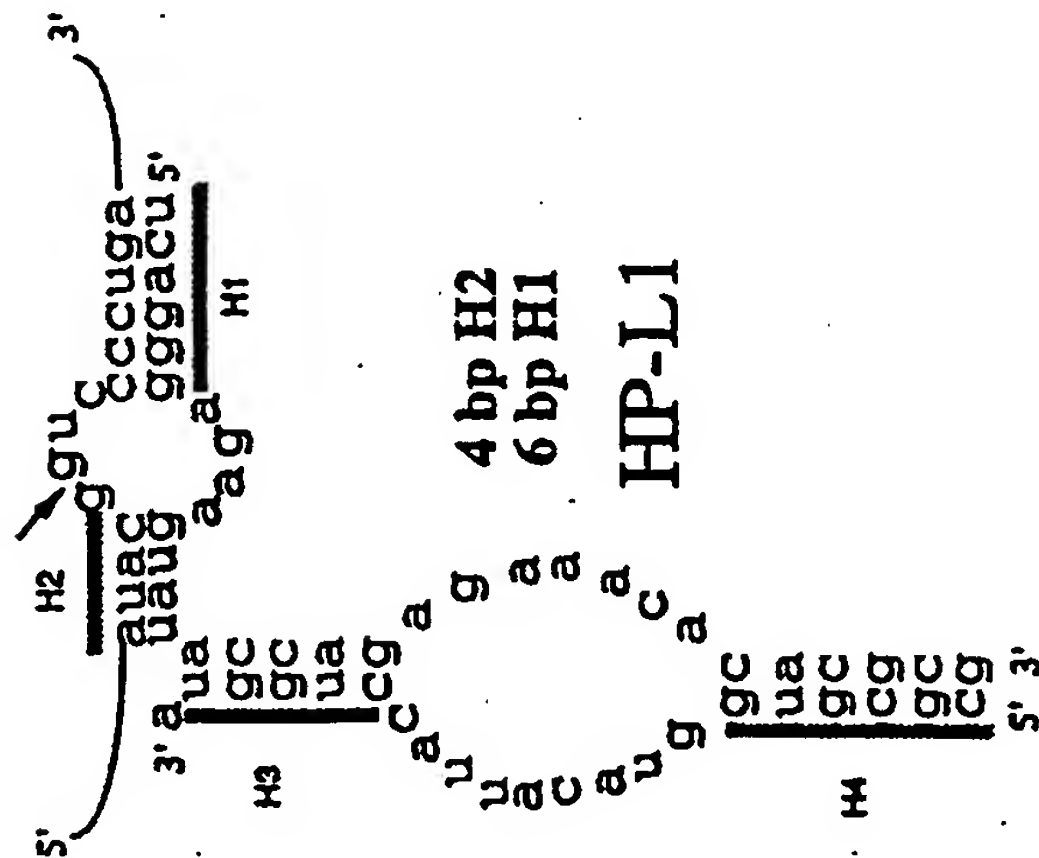


FIG. 69a.

Substrate RNA



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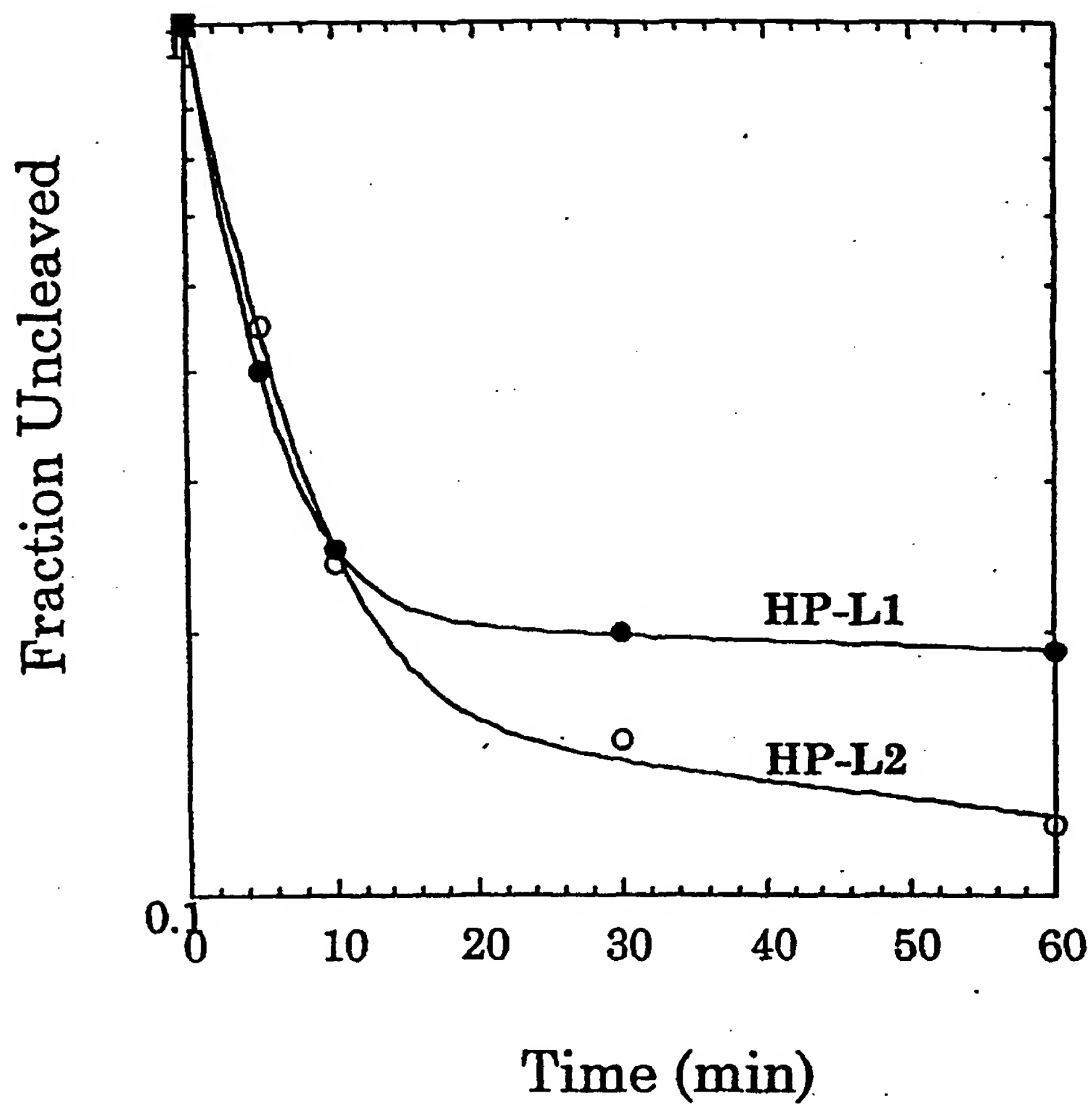


FIG. 70.

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FIG. 71b.

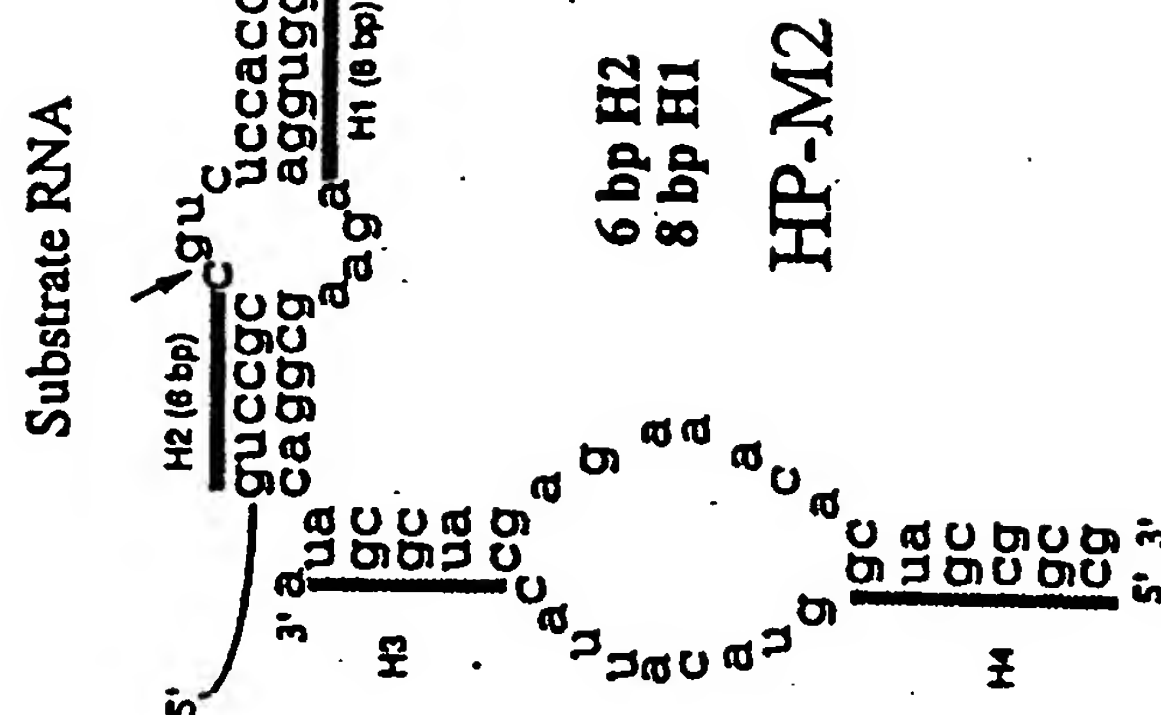
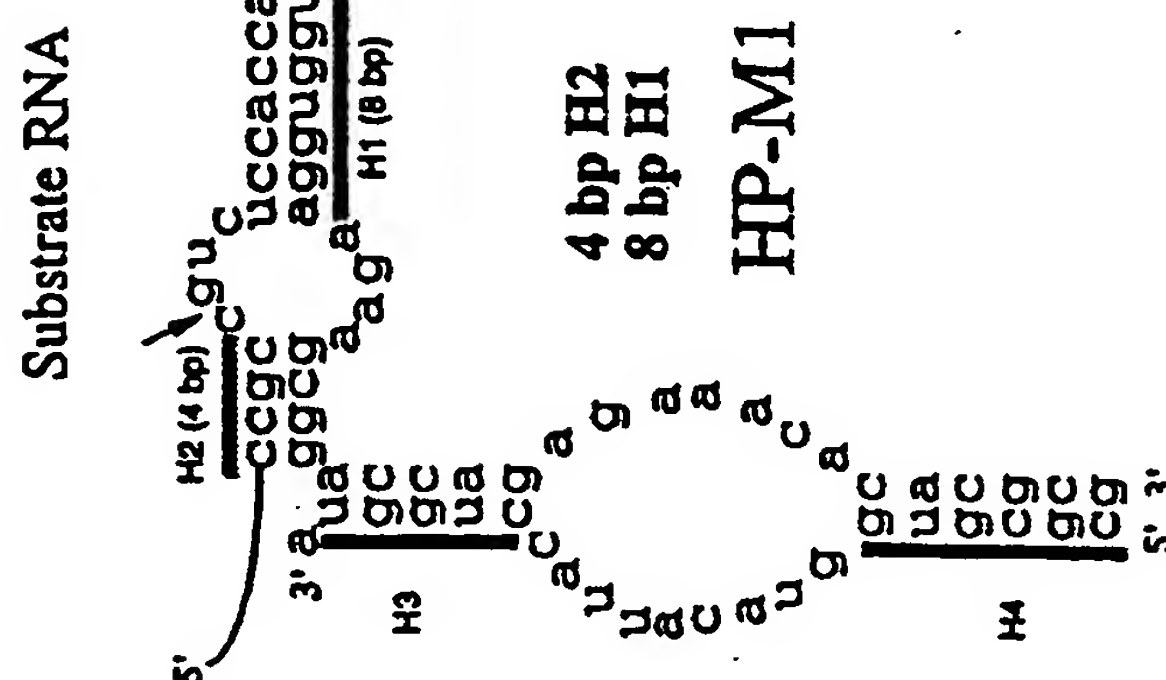


FIG. 71a.



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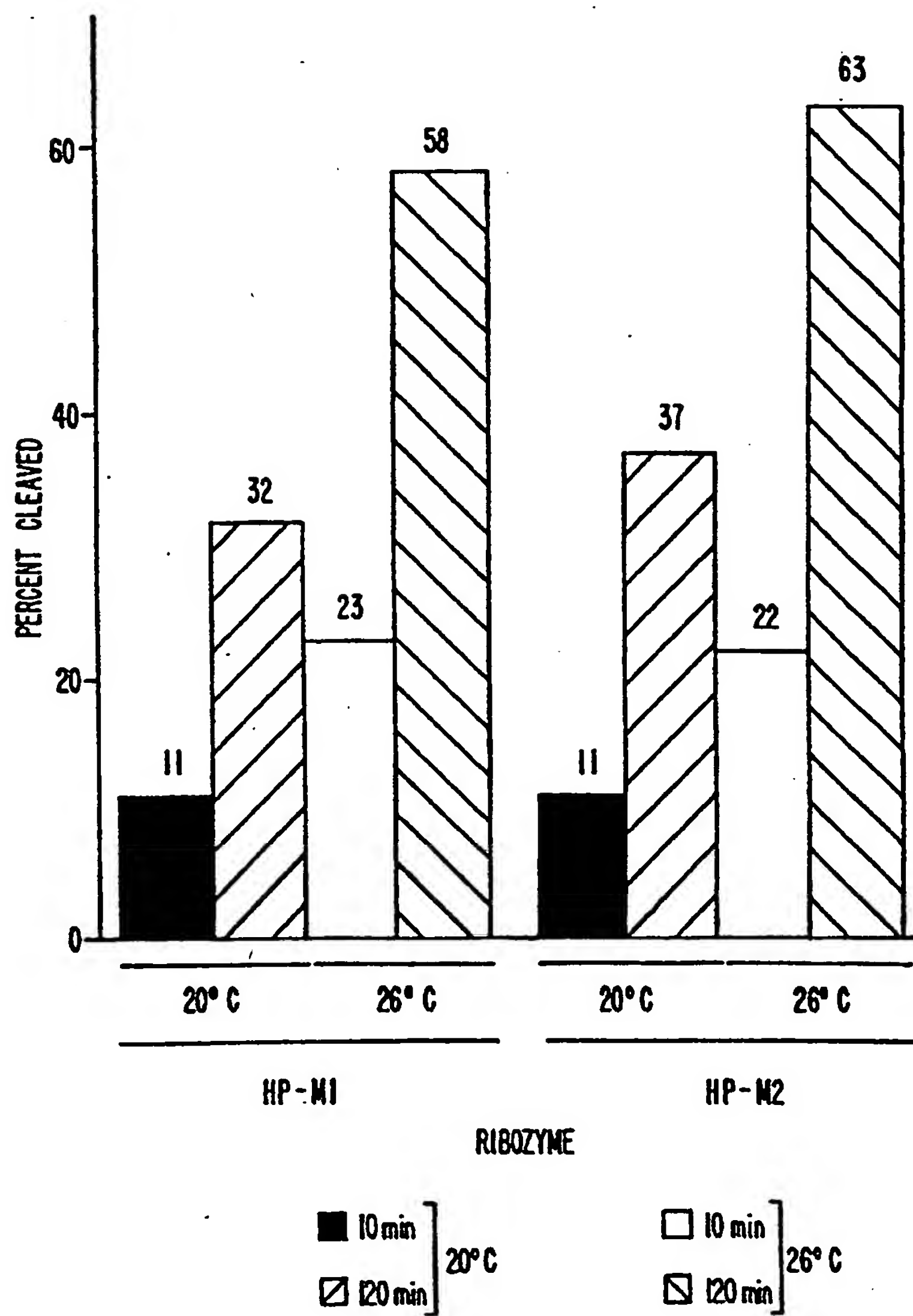


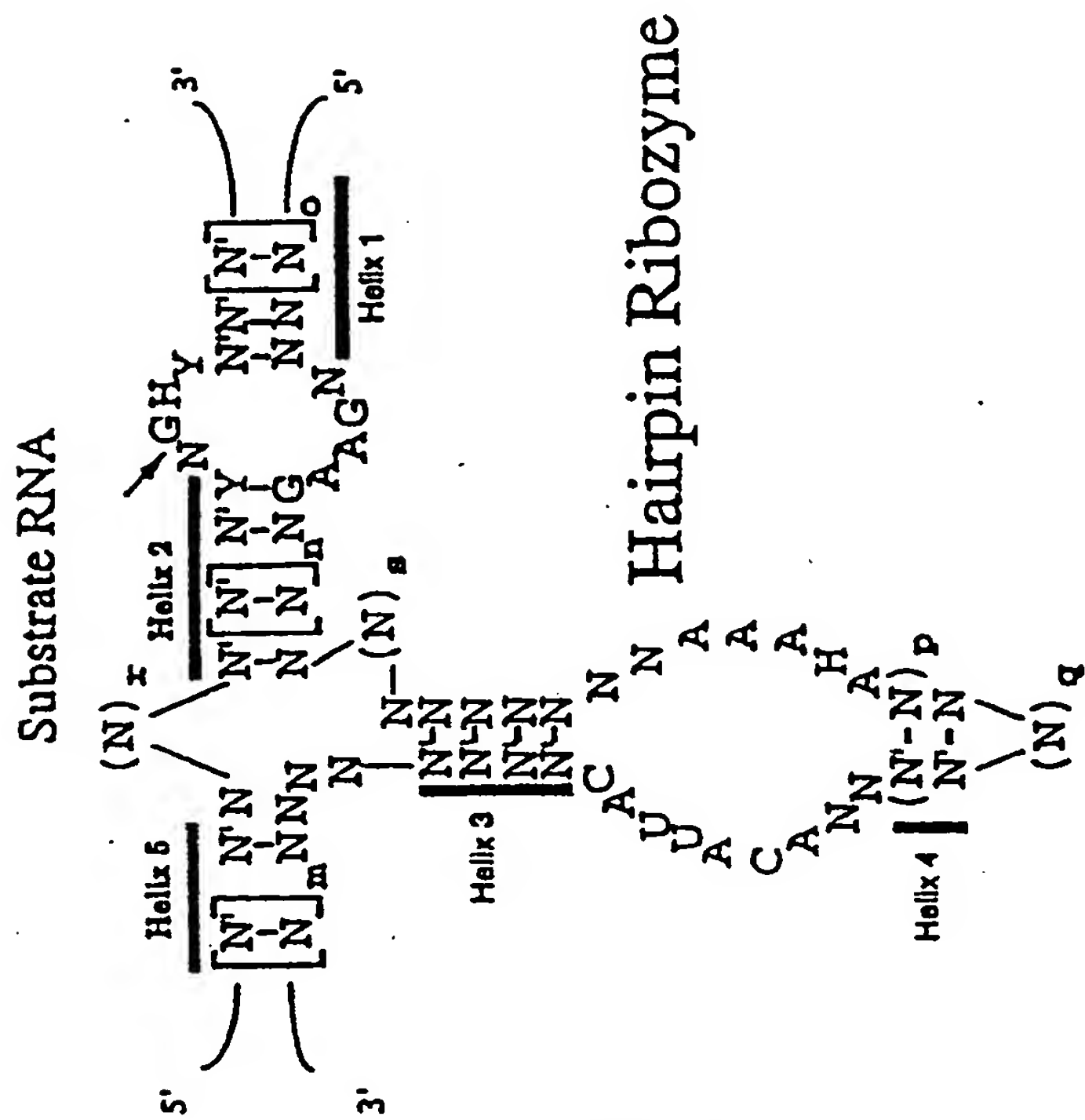
FIG. 72.

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FIG. 74a.

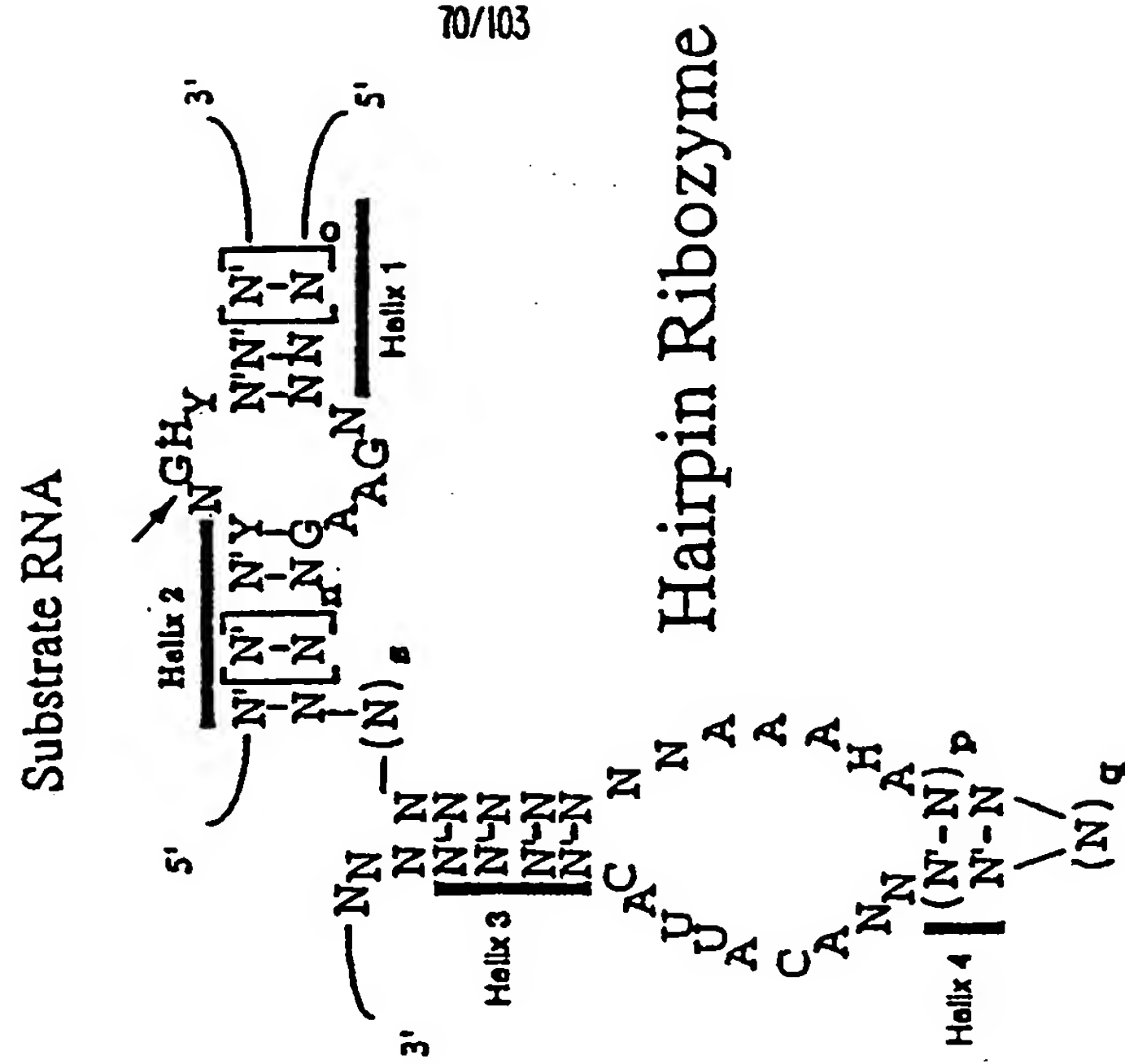
A



SUBSTITUTE SHEET (RULE 26)

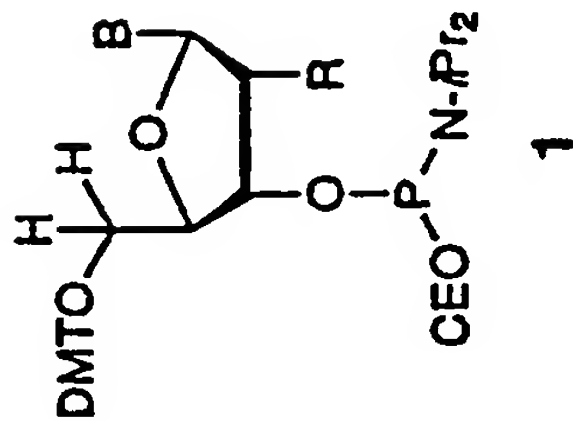
FIG. 74b.

B



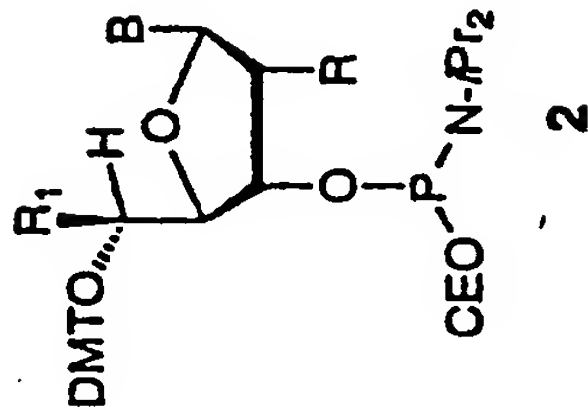
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FIG. 75a.



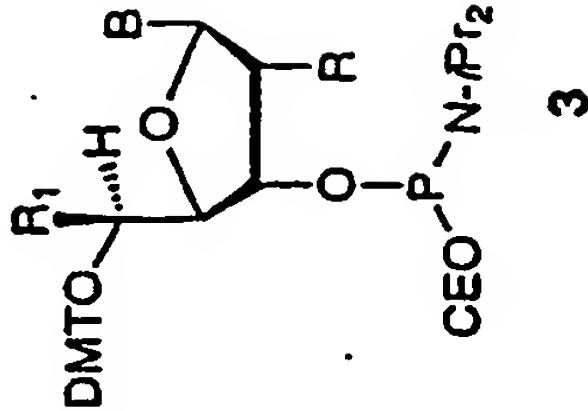
D-Ribose Family

FIG. 75b.



D-Allose Family

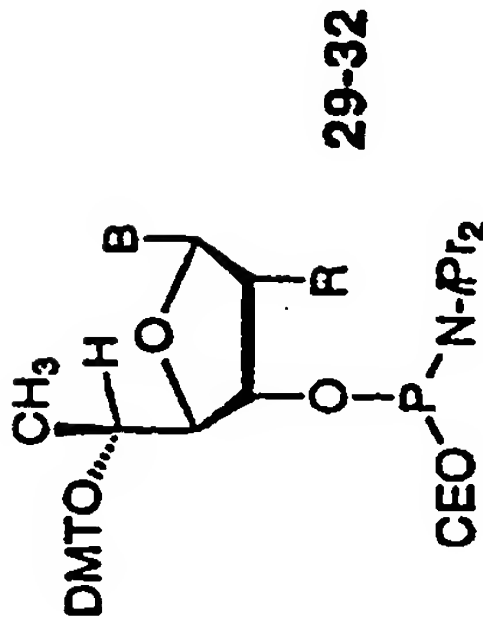
FIG. 75c.



L-Talose Family

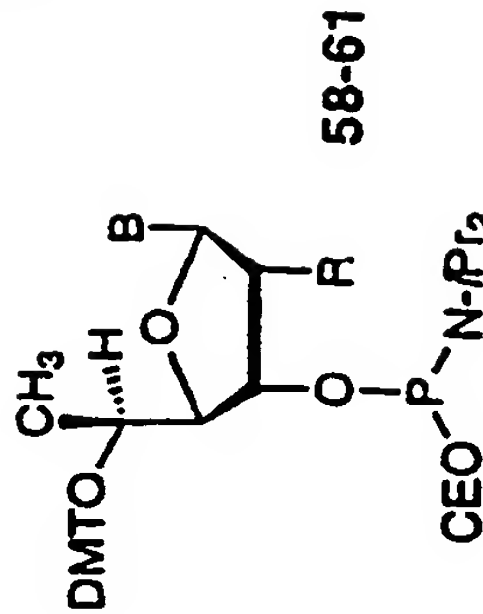
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FIG. 75d.



D-Allose

FIG. 75e.



L-Talose

B = Protected A, C, G, U, T, 2AP, I, DIAP, P etc.

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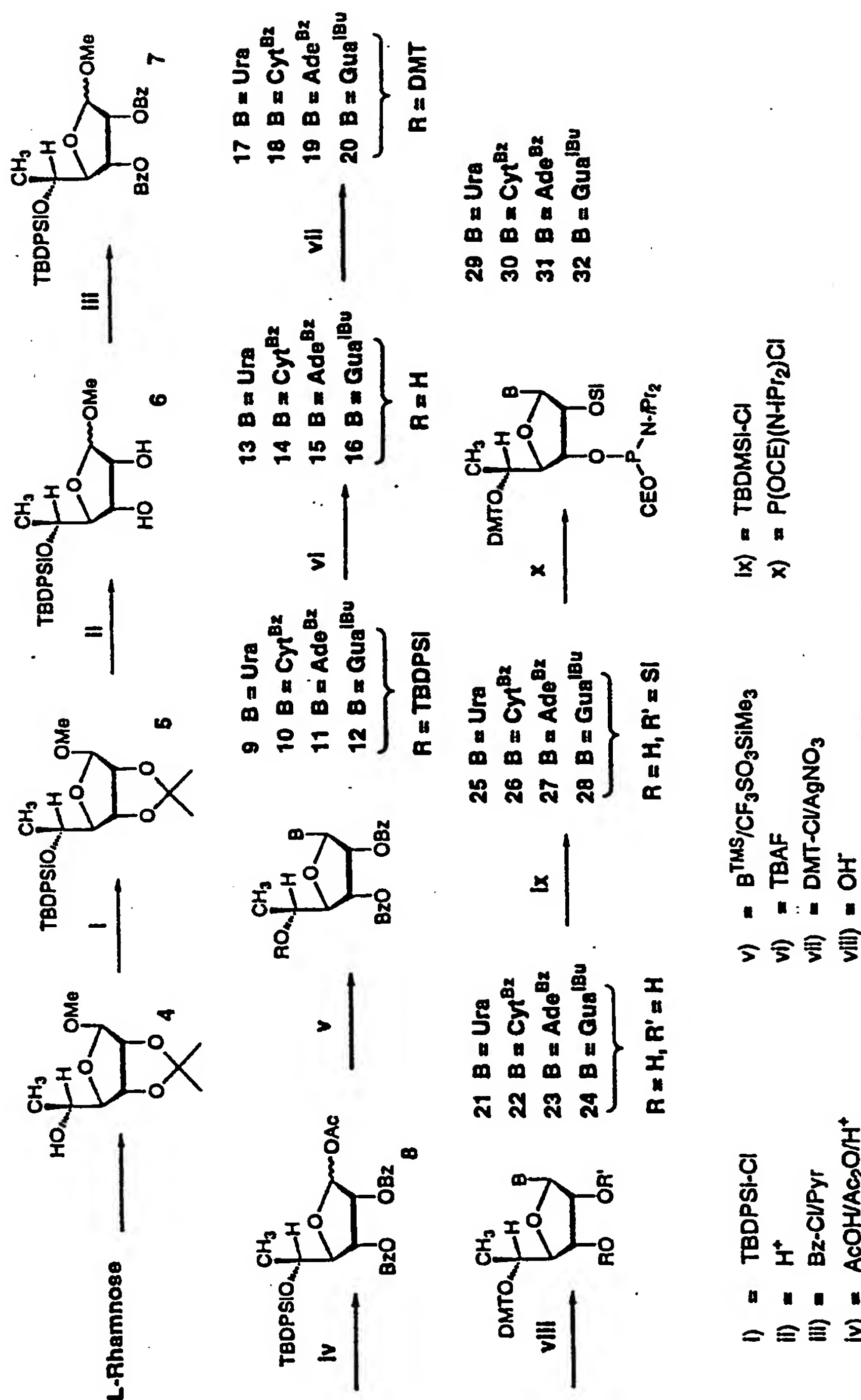
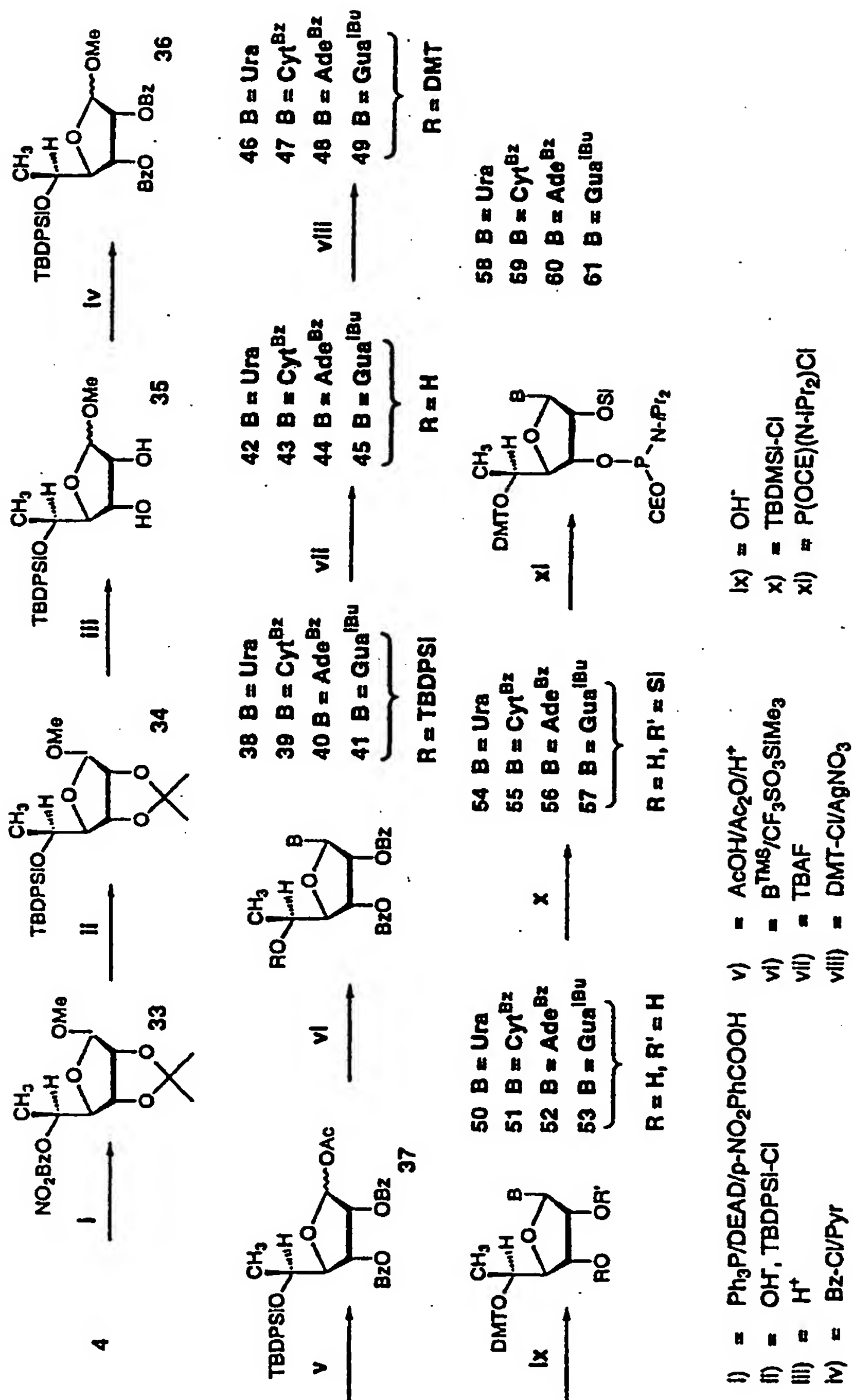


FIG. 76.

SUBSTITUTE SHEET (RULE 26)

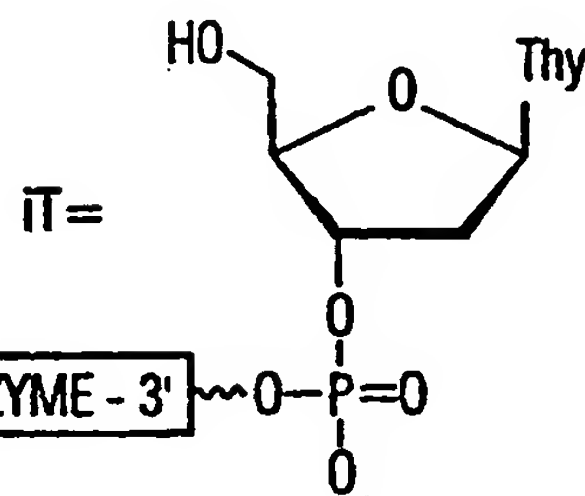
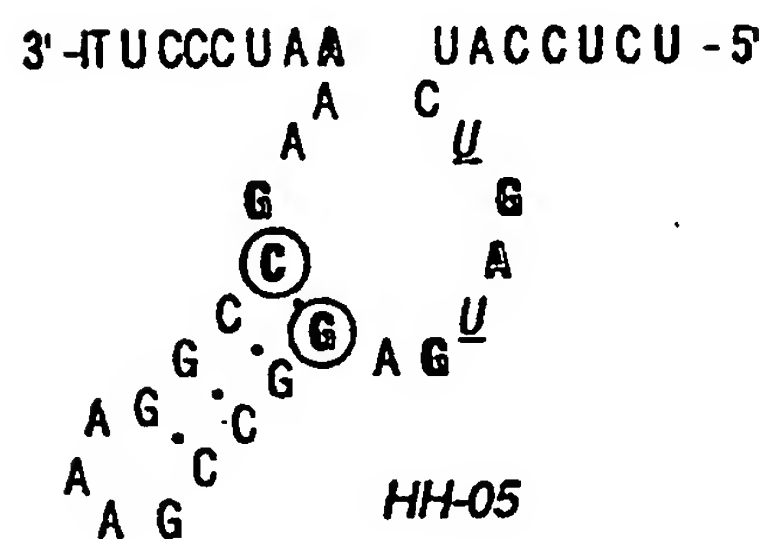
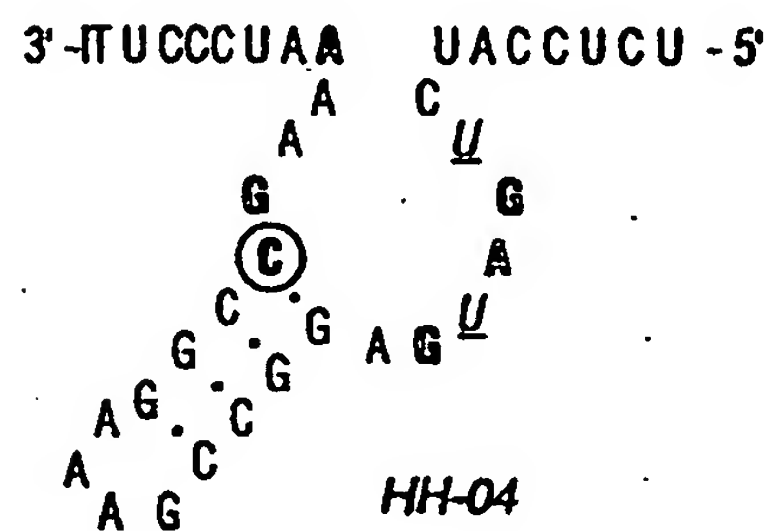
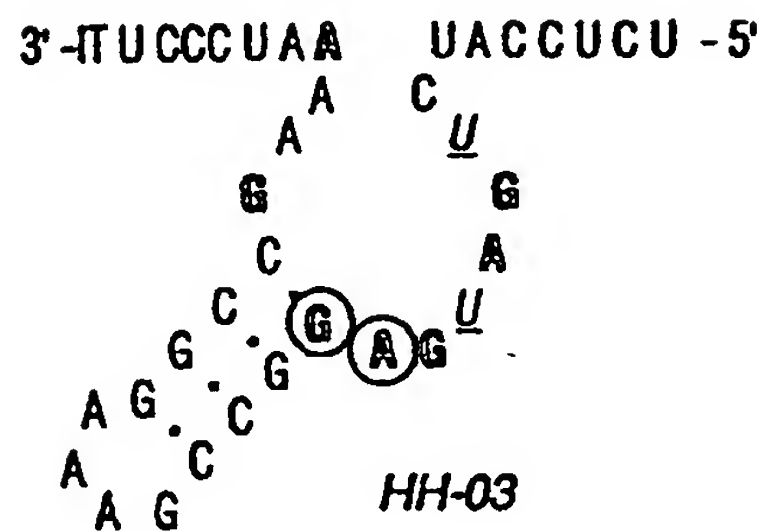
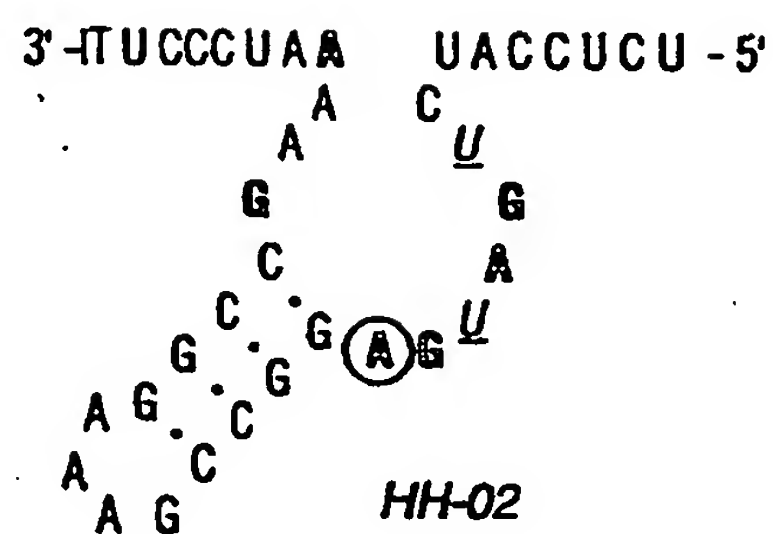
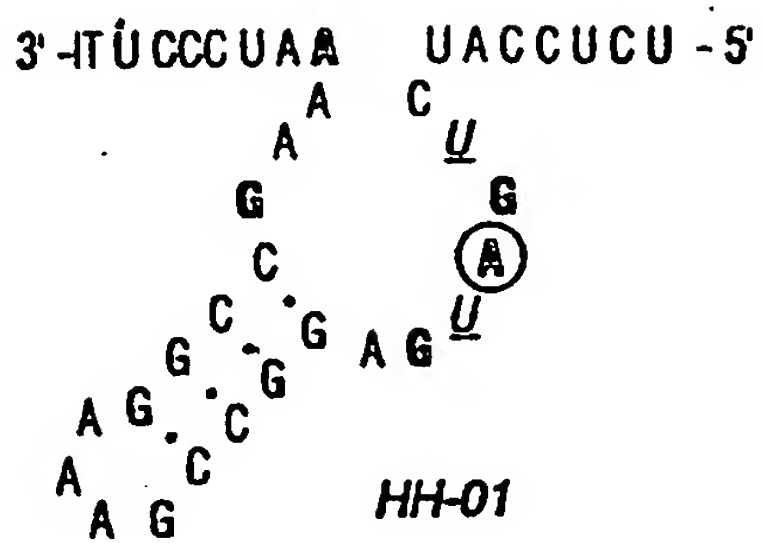
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FIG. 78.



N=2'-O-Me

N=RIBO

U=2'-NH₂U

(N)=TALO

WHERE THE ALPHABET "N" REPRESENTS A NUCLEOTIDE, A, U, G, OR C
SUBSTITUTE SHEET (RULE 26)

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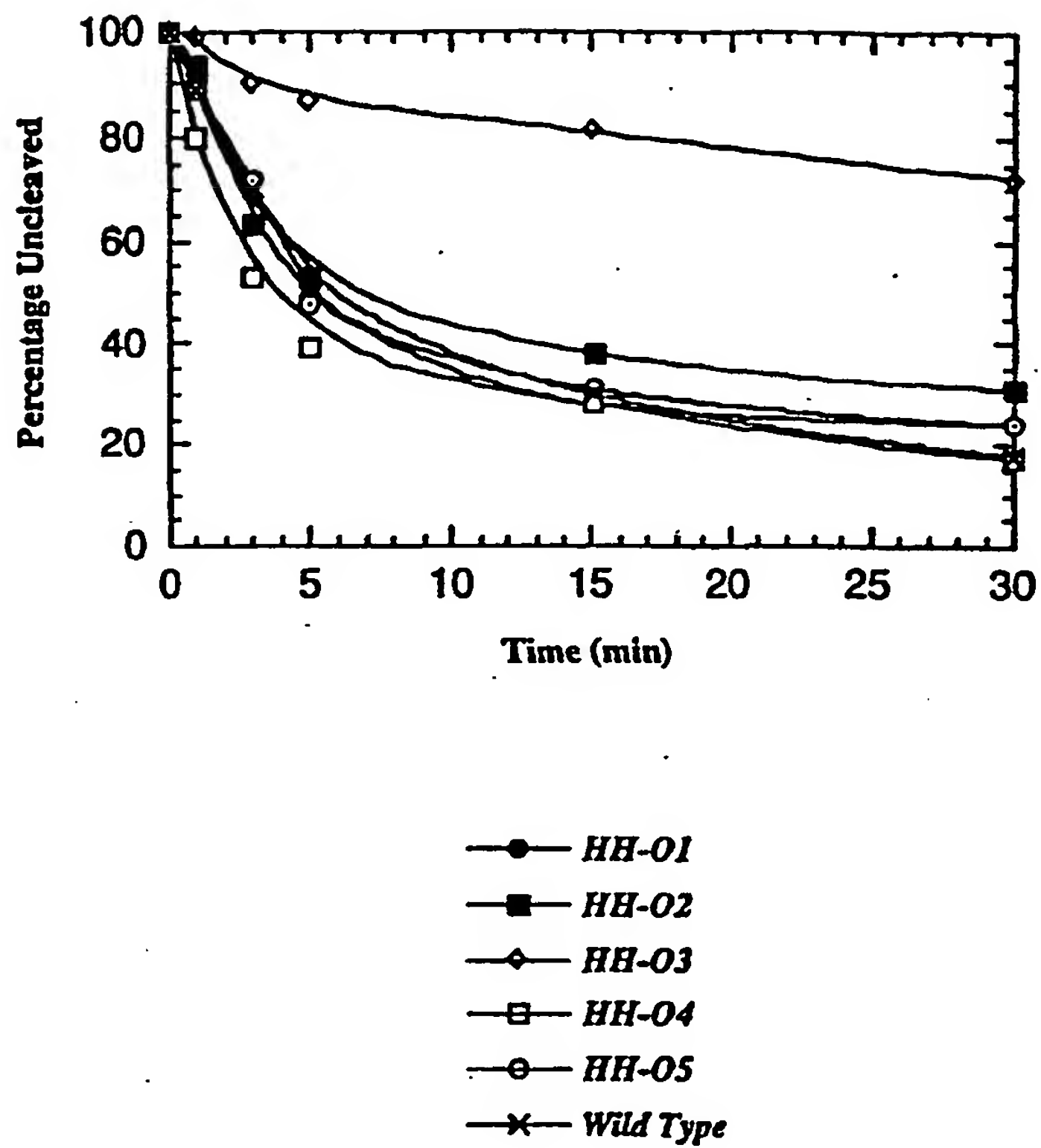
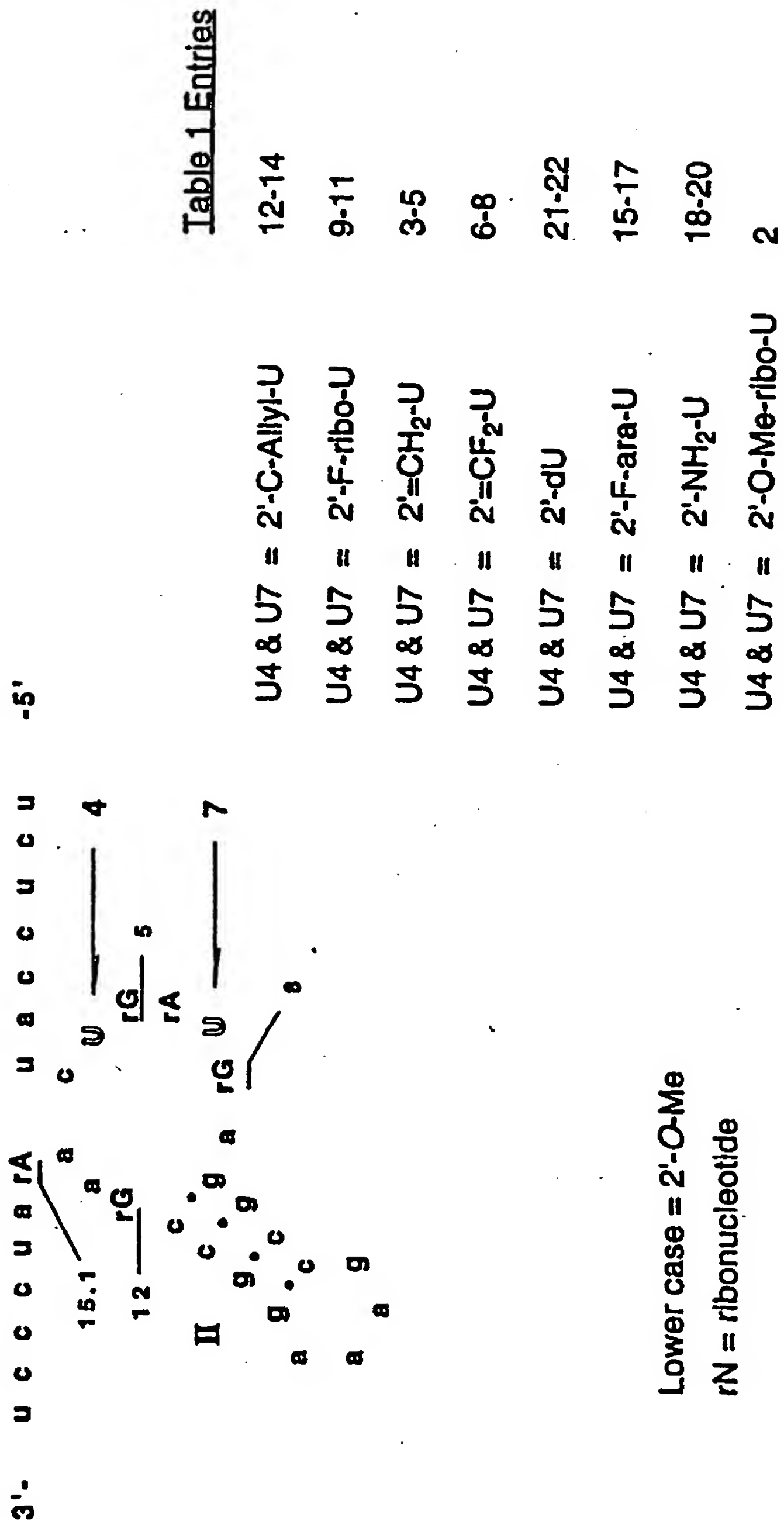


FIG. 79.

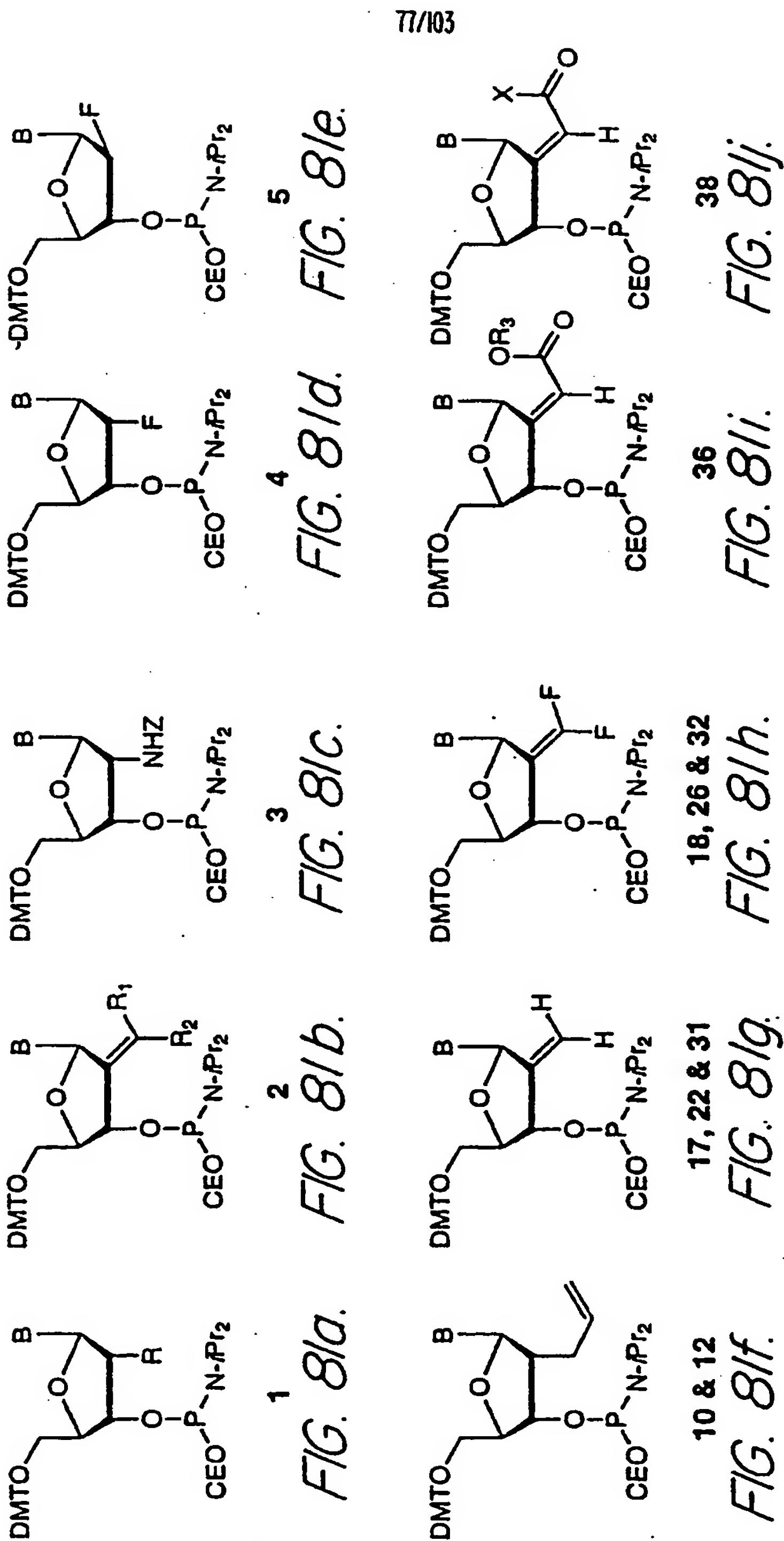
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Lower case = 2'-O-Me
rN = ribonucleotide

FIG. 80.



SUBSTITUTE SHEET (RULE 26)

B = Protected A, C, G, U, T, 2AP, I, DIAP, P etc.

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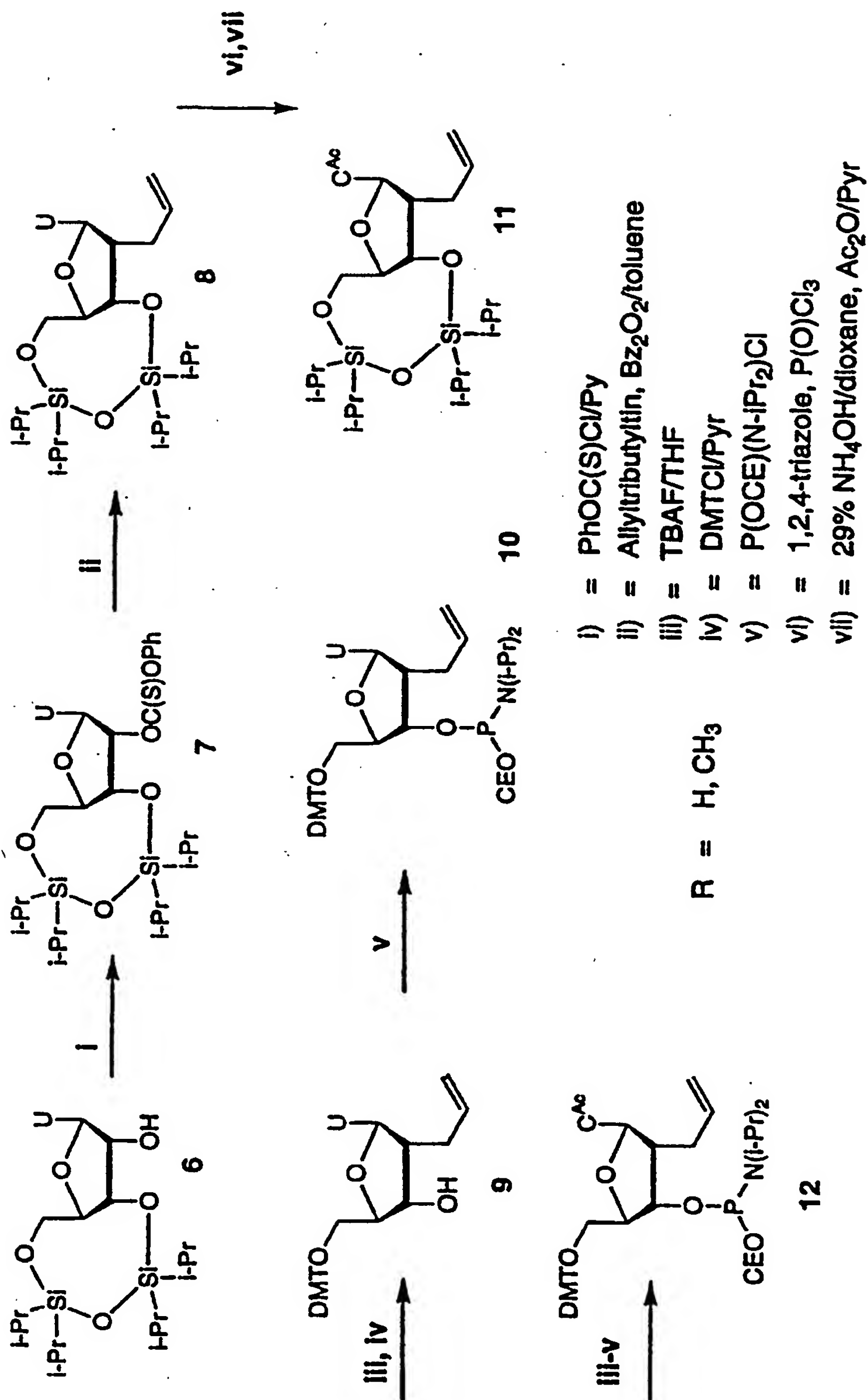
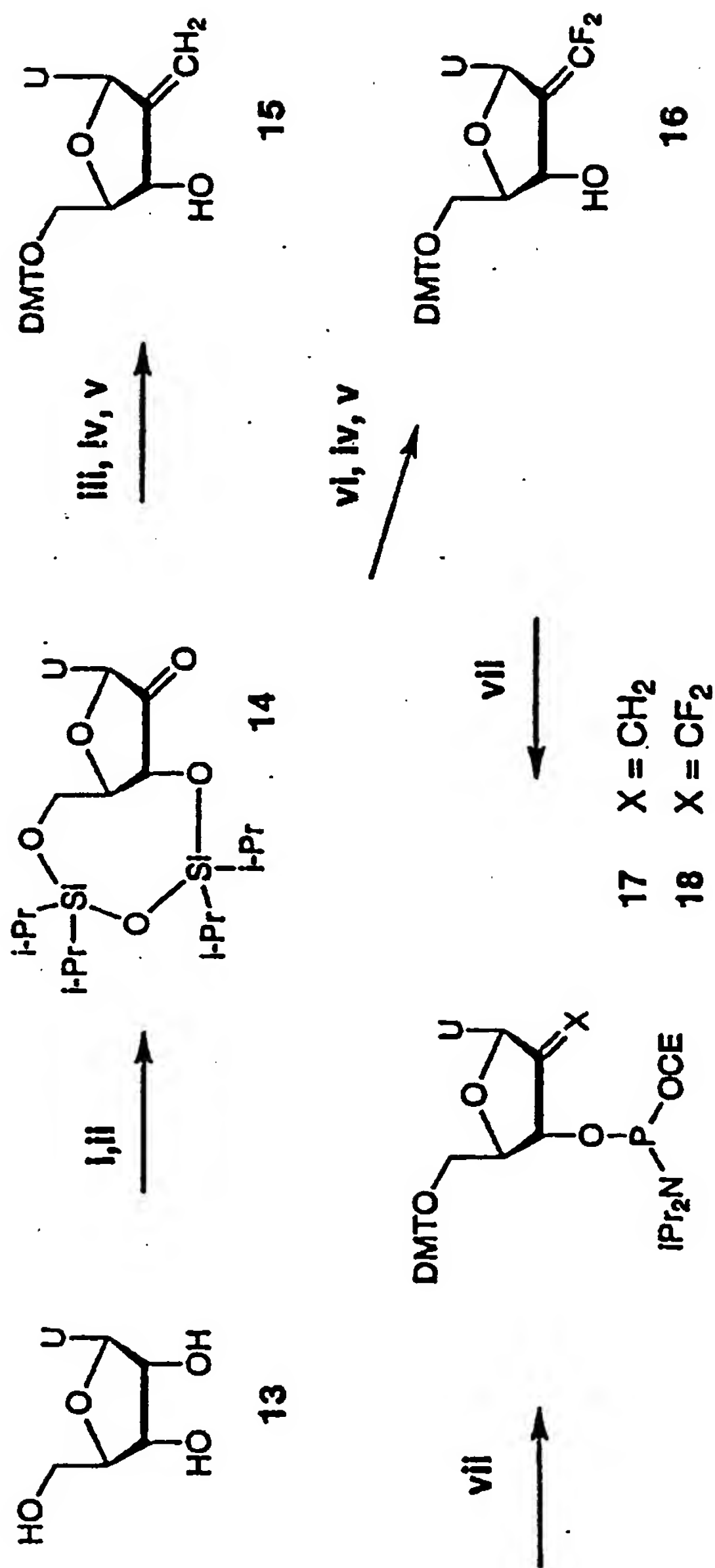


FIG. 82.

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FIG. 83.

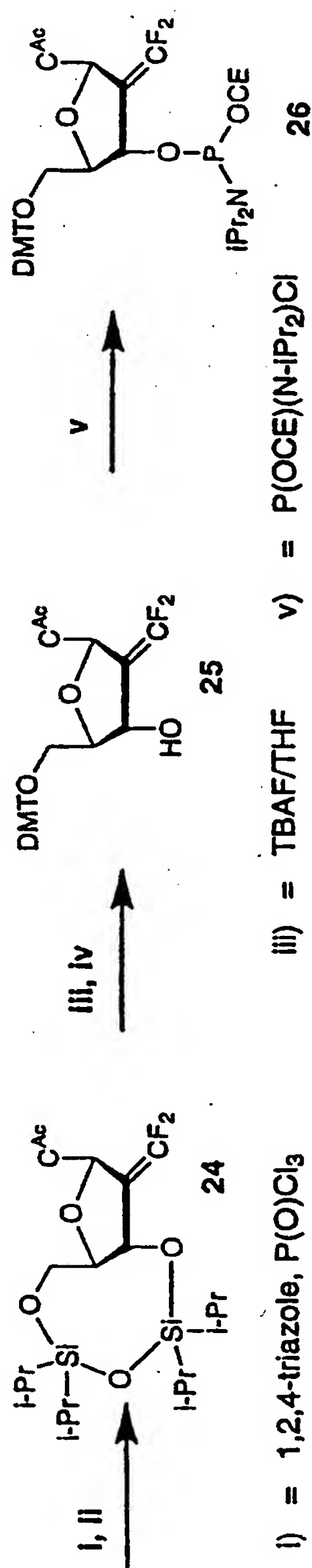
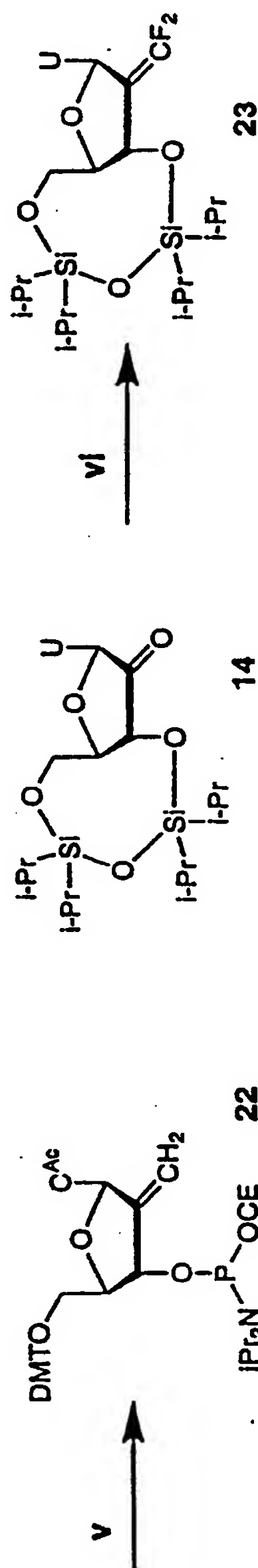
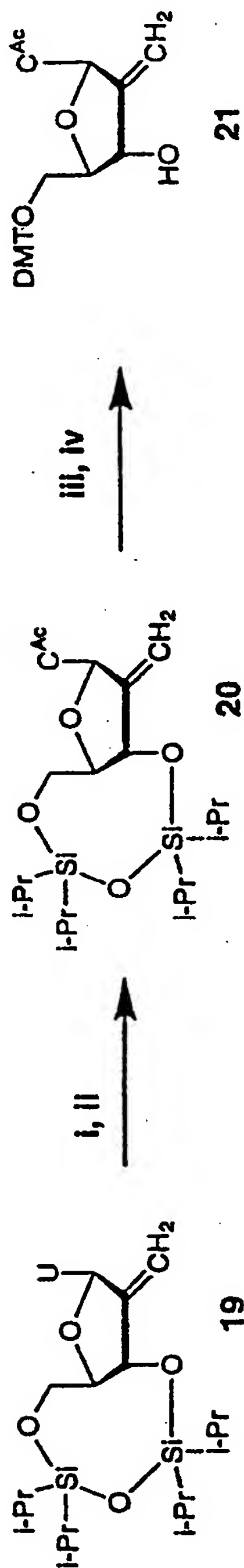


- i) = Markiewicz reagent
 ii) = DMSO & Ac₂O
 iii) = Ph₃PCH₃I
 iv) = TBAF/THF
 v) = DMTCI/Pyr
 vi) = Ph₃P, ClCF₂COONa
 vii) = P(OCE)(N-iPr₂)Cl

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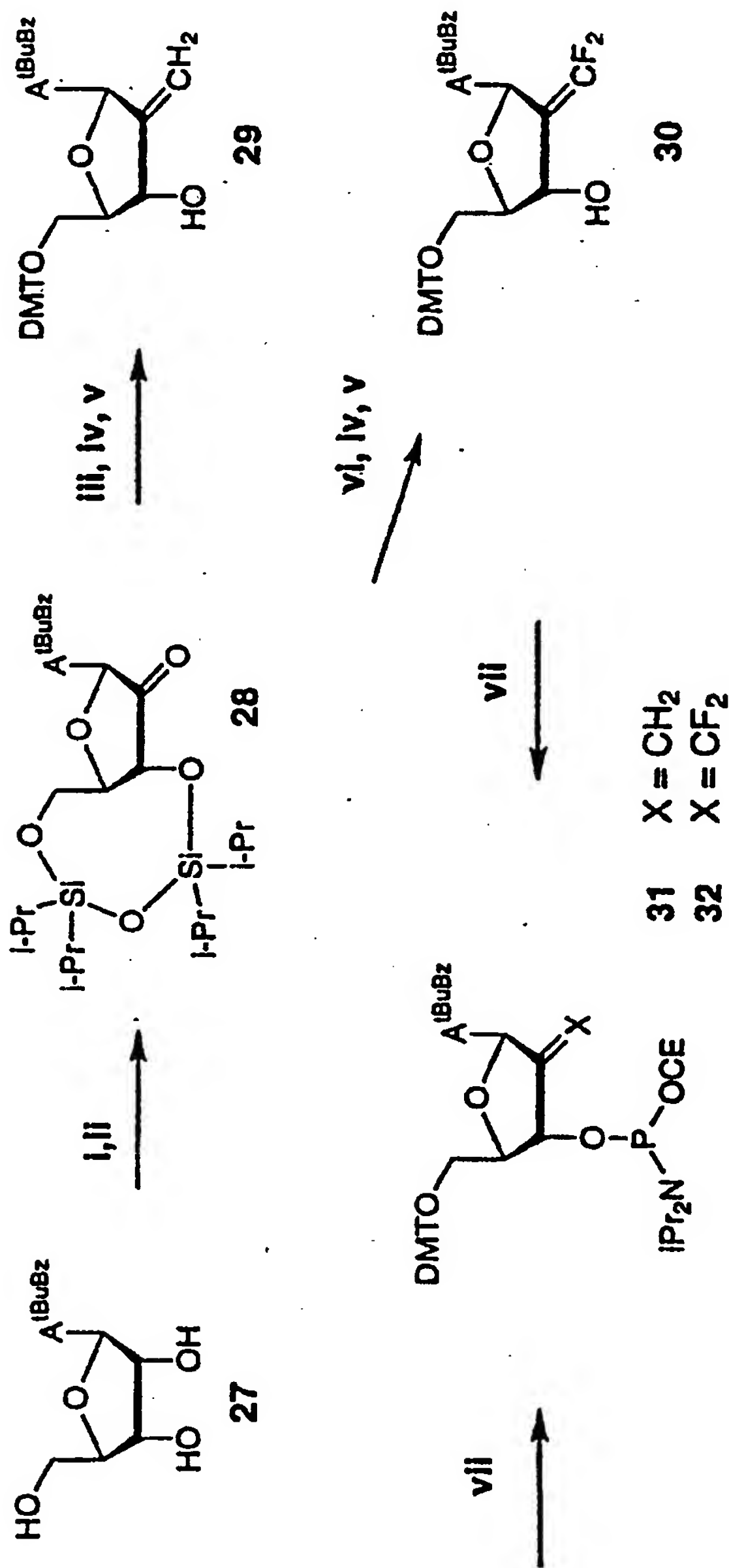
FIG. 84.



i) = 1,2,4-triazole, $\text{P}(\text{O})\text{Cl}_3$
 ii) = 29% NH_4OH /dioxane, Ac_2O /Pyr
 iii) = TBAF/THF
 iv) = DMTCI /Pyr
 v) = $\text{P}(\text{OCE})(\text{N-iPr}_2)\text{Cl}$
 vi) = Ph_3P , $\text{ClCF}_2\text{COONa}$

SUBSTITUTE SHEET (RULE 26)

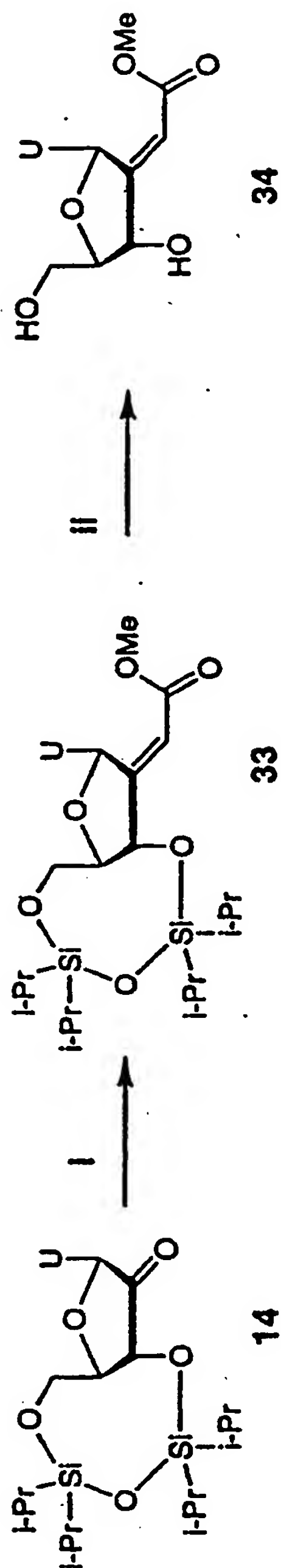
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- | | |
|---|--|
| i) = Markiewicz reagent | v) = DMTCI/Pyr |
| ii) = DMSO & Ac ₂ O | vi) = Ph ₃ P, ClCF ₂ COONa |
| iii) = Ph ₃ PCH ₃ I | vii) = P(OCE)(N-IPr ₂)Cl |
| iv) = TBAF/THF | |

FIG. 85.

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- i) = $\text{Ph}_3\text{PC}=\text{CHC}(\text{O})\text{OCH}_3\cdot\text{OAc}$
 ii) = $\text{NEt}_3\cdot 3\text{HF}$
 iii) = DMTCI/Pyr
 iv) = $\text{P}(\text{OCE})(\text{N-IPr}_2)\text{Cl}$
 v) = MeOH/NaOH

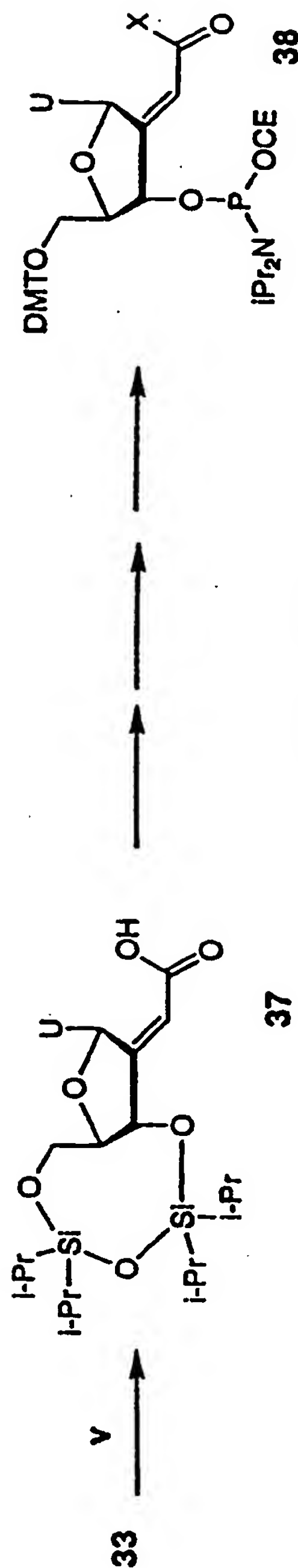
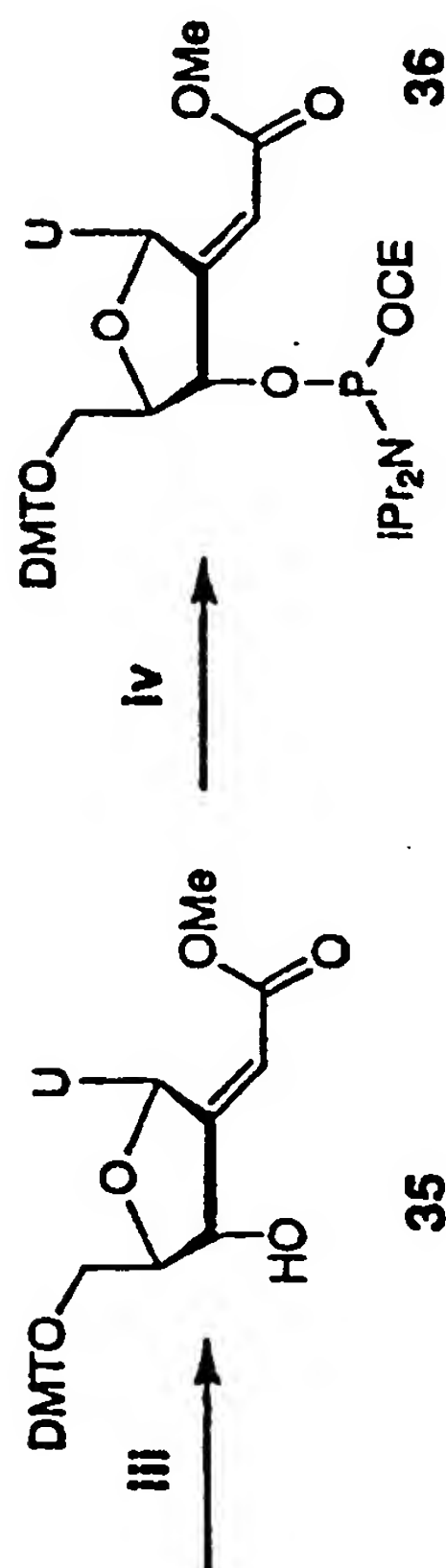
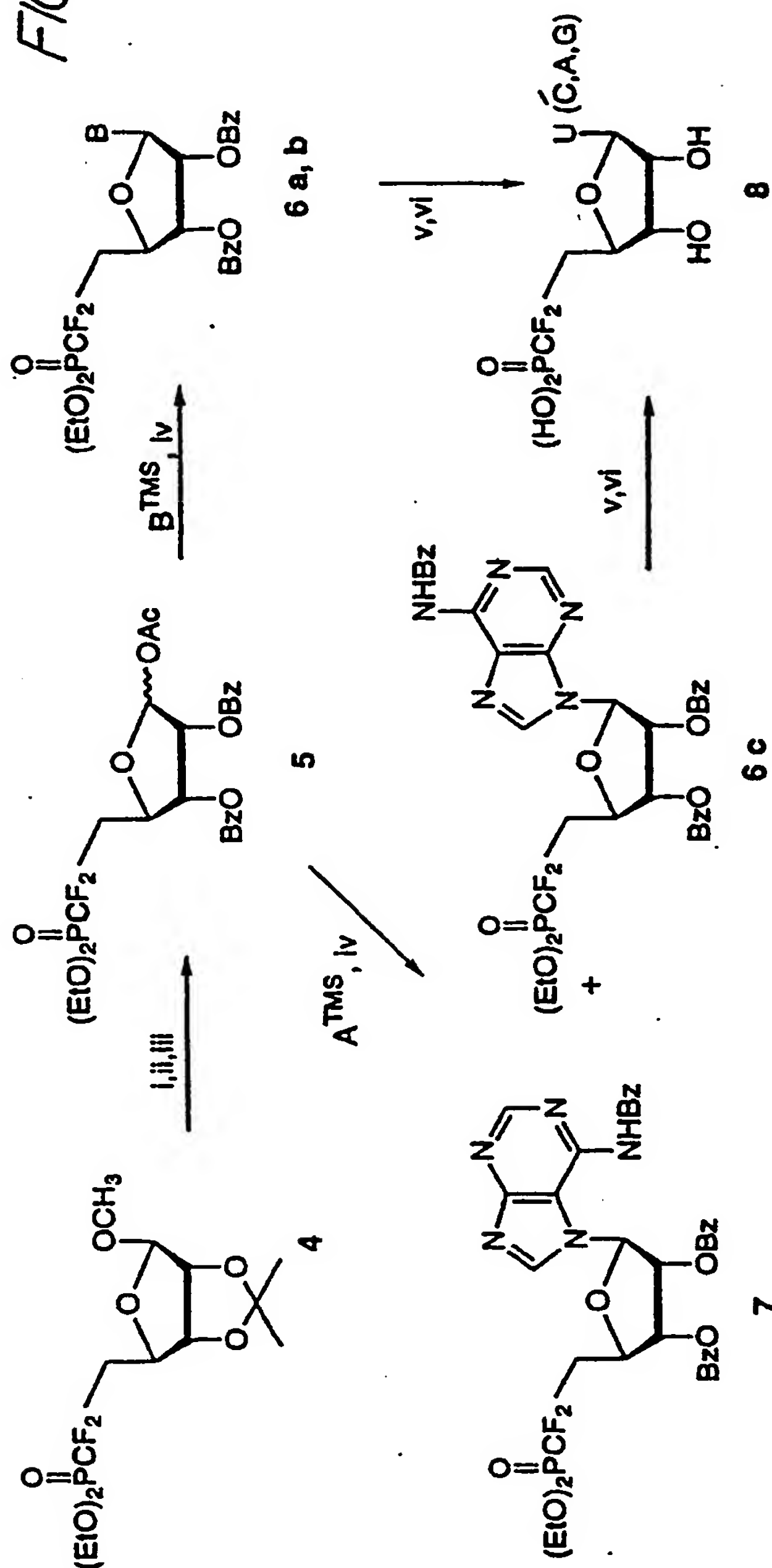


FIG. 86.

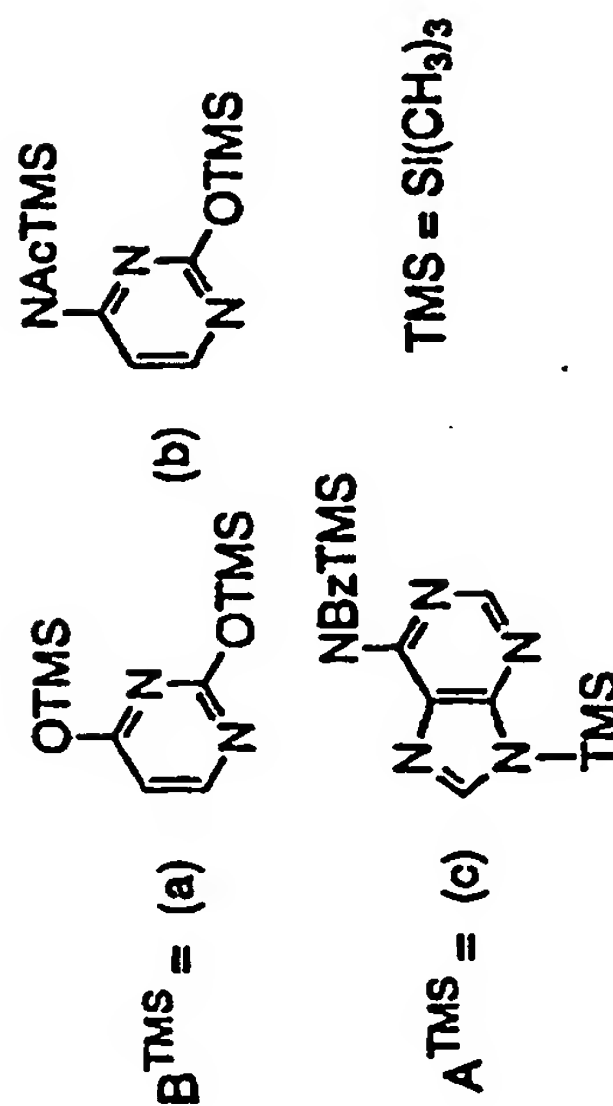
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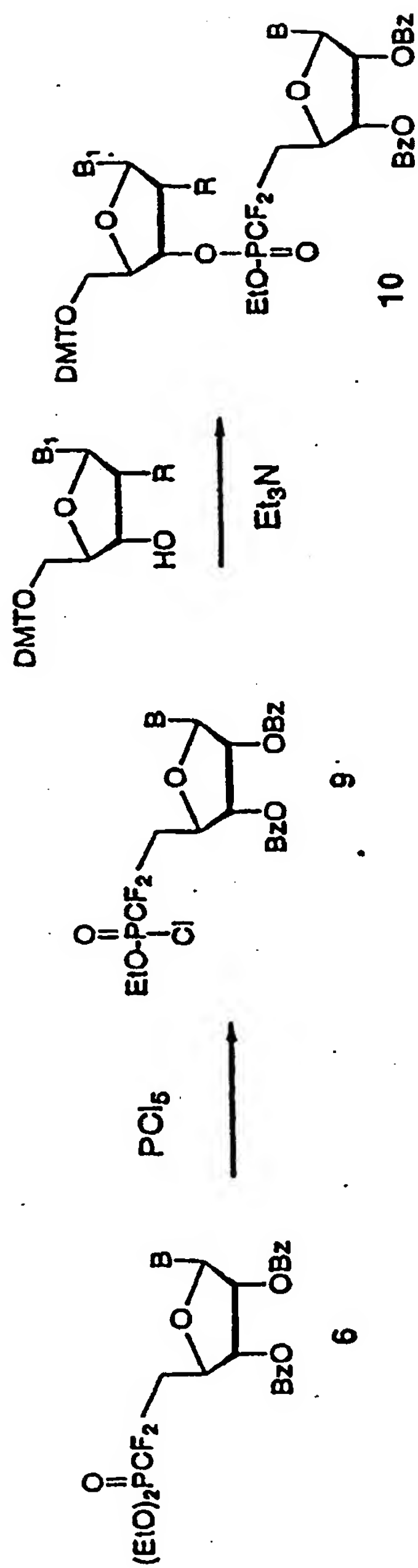
FIG. 87.



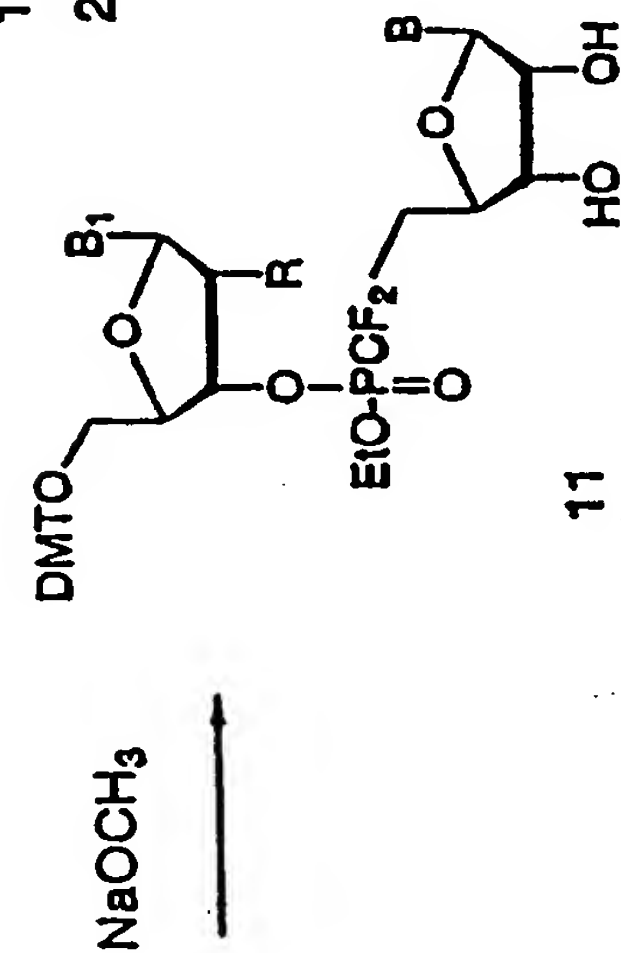
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1. TBDMS-Cl
2. a. (CEO)P(Cl)N IP_r₂
b. polymeric support



B, B₁ = uracil, N-Z-cytosine, N-Z-adenine, N-Z-guanine etc.

Z = amino-protecting group

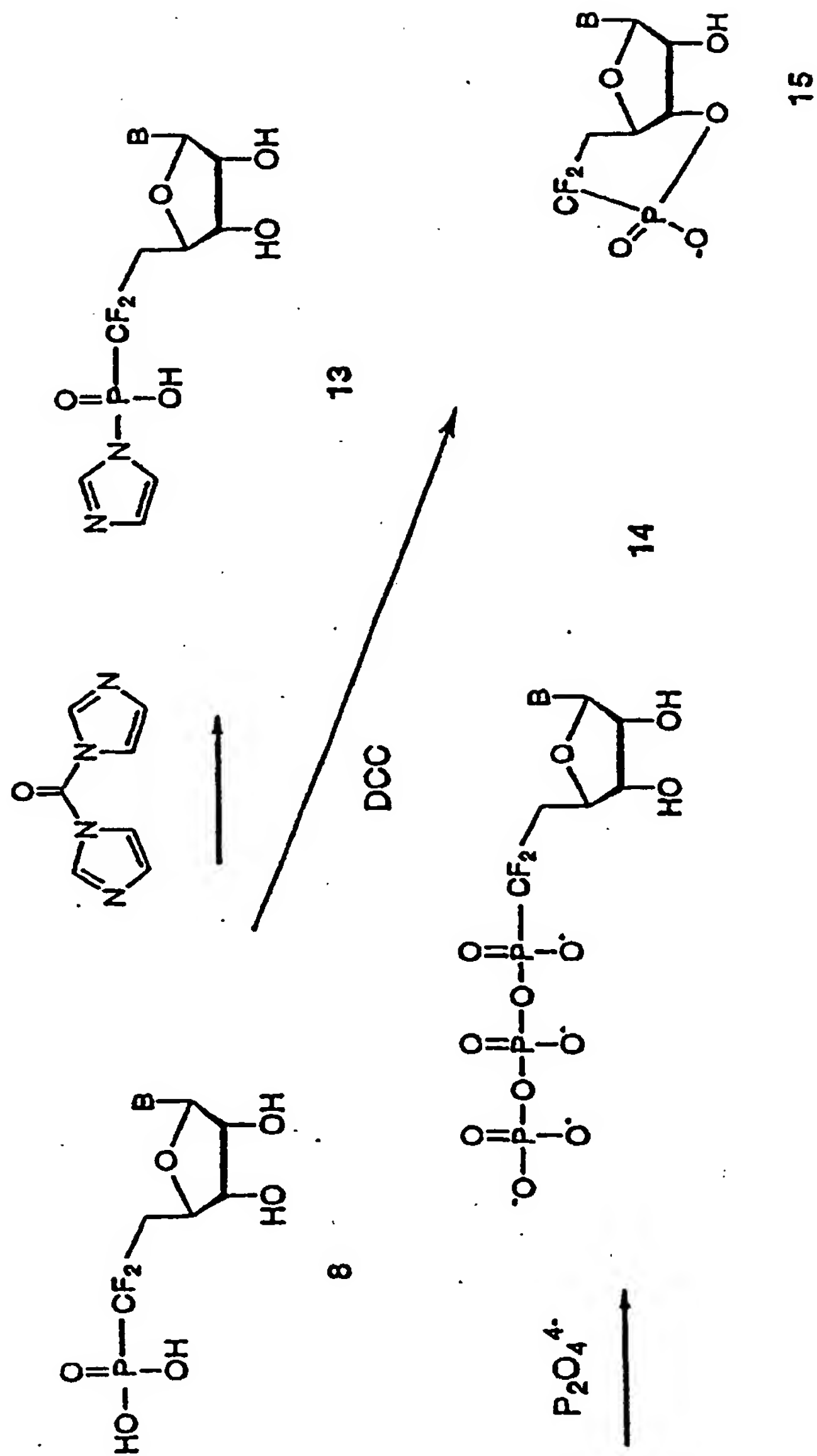
R = OTBDMS, OCH₃, H

a. X = P(OCE)N IP_r₂
X = polymeric support

FIG. 88.

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B = uracil, N-Z-cytosine, N-Z-adenine, N-Z-guanine etc.

Z = amino-protecting group

FIG. 89.

SUBSTITUTE SHEET (RULE 26)

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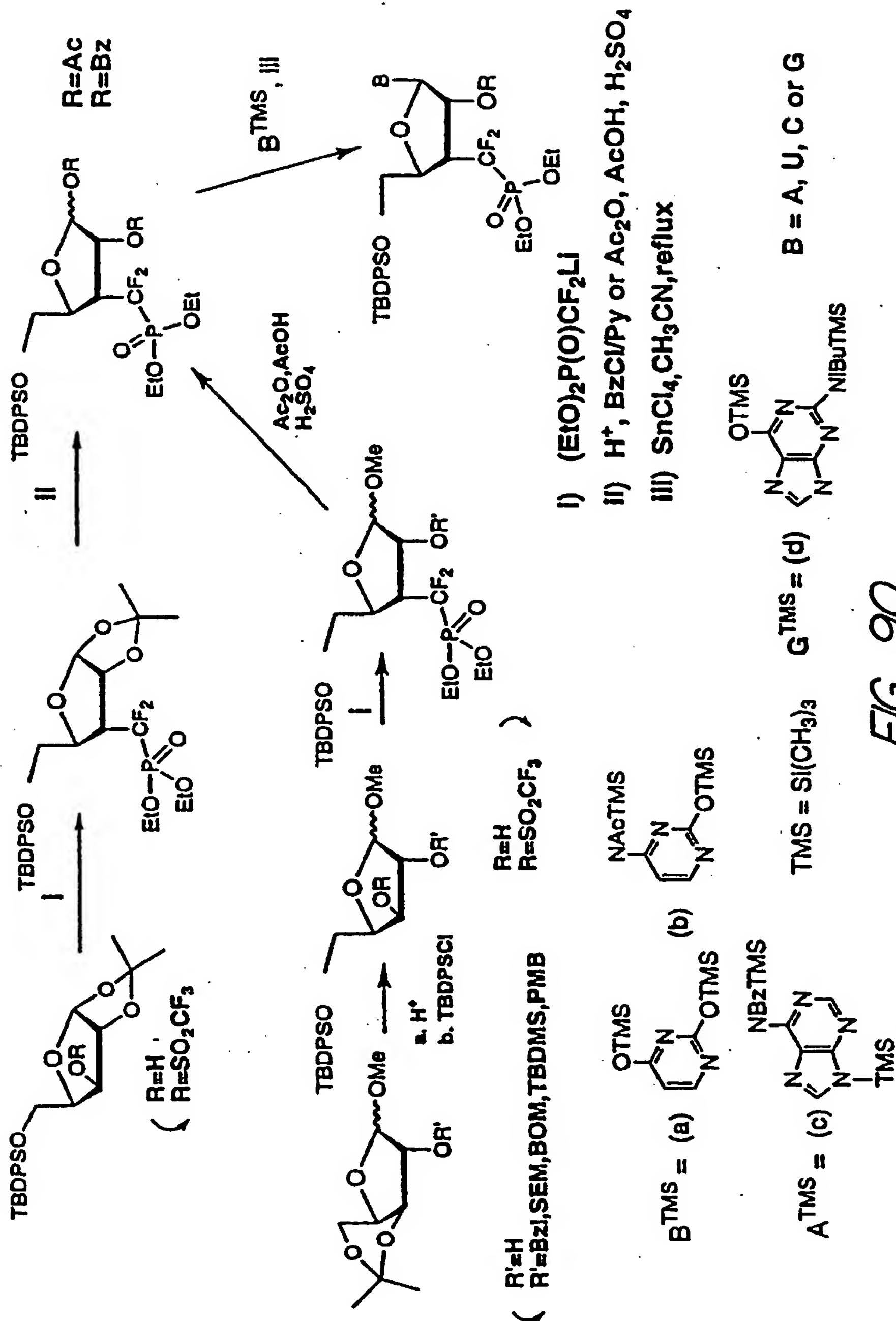


FIG. 90.

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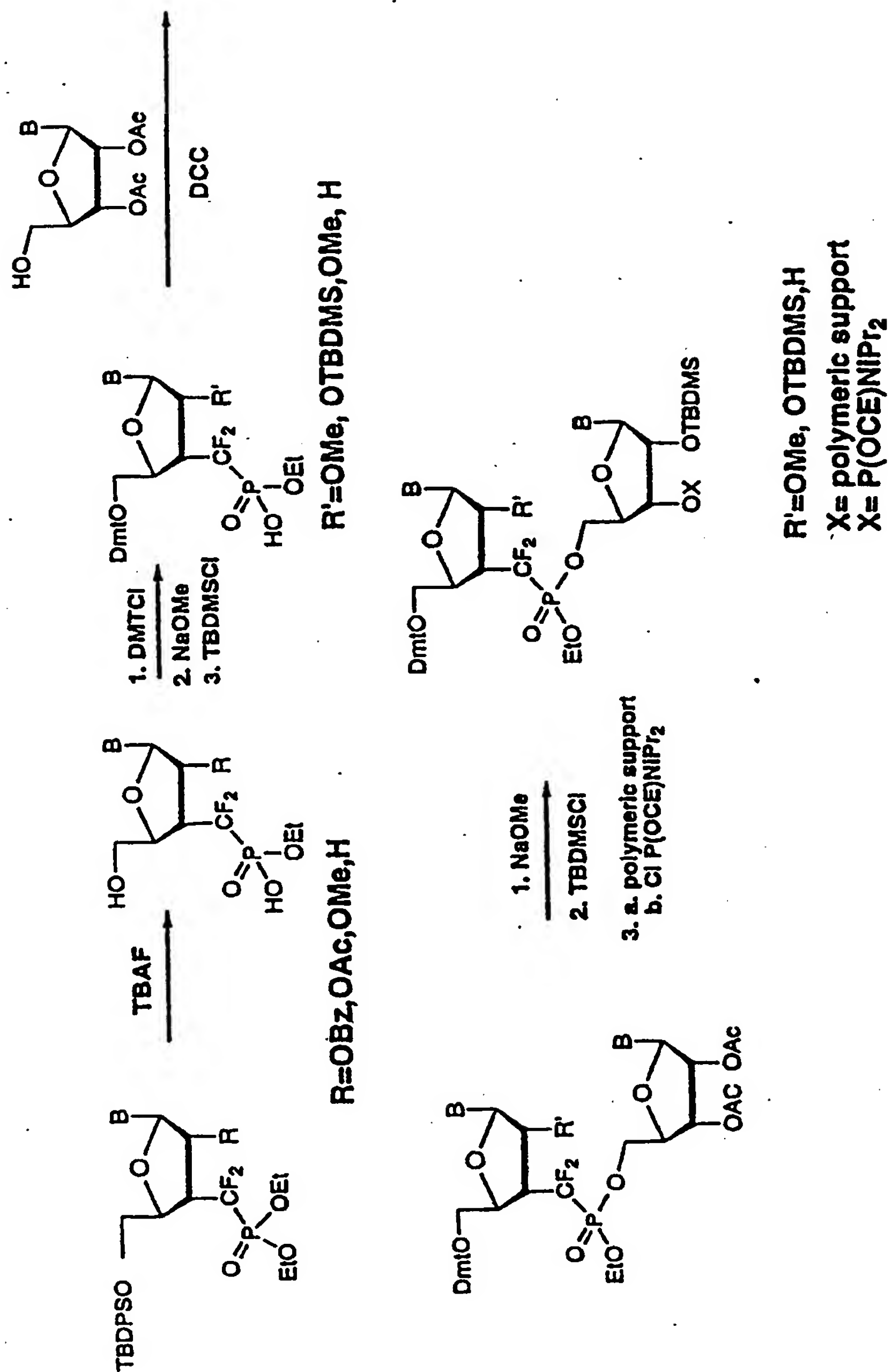
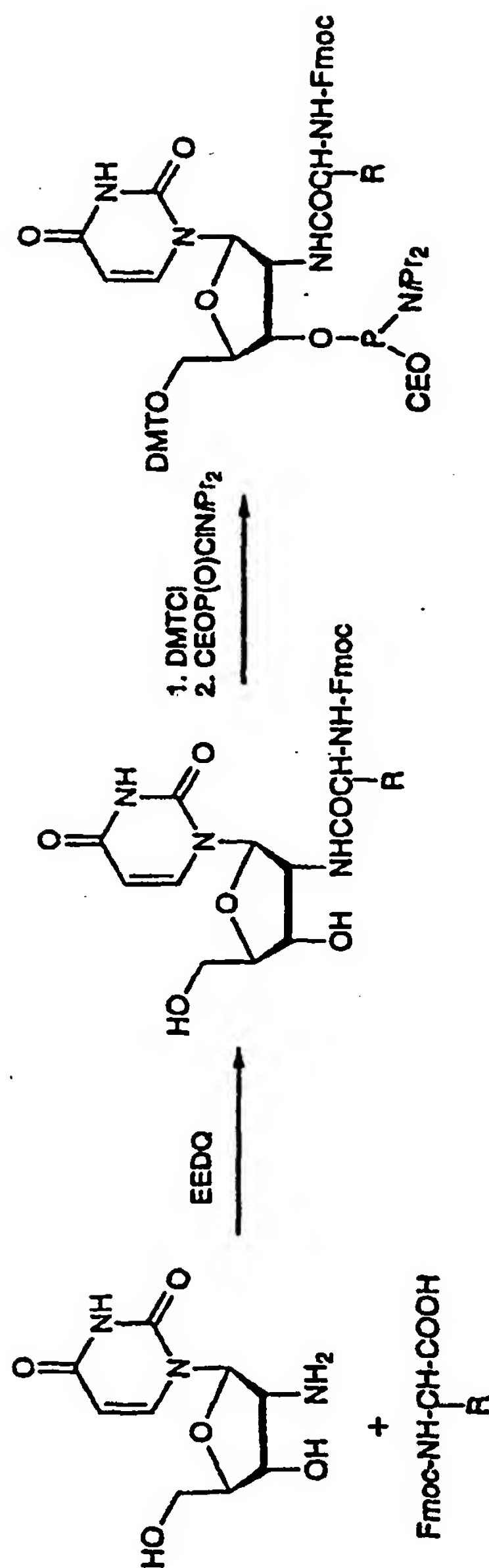
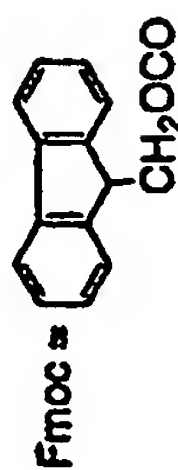


FIG. 91.

SUBSTITUTE SHEET (RULE 26)



EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline



R = CH₃, CH₂-(phe), (CH₂)₄NH-Fmoc, (CH₂)₄NH-CBZ, CH₂COOBzl (ala), (lys), (asp)

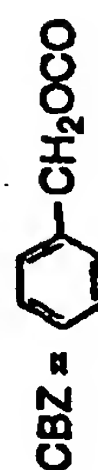


FIG. 92.

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FIG. 93a.

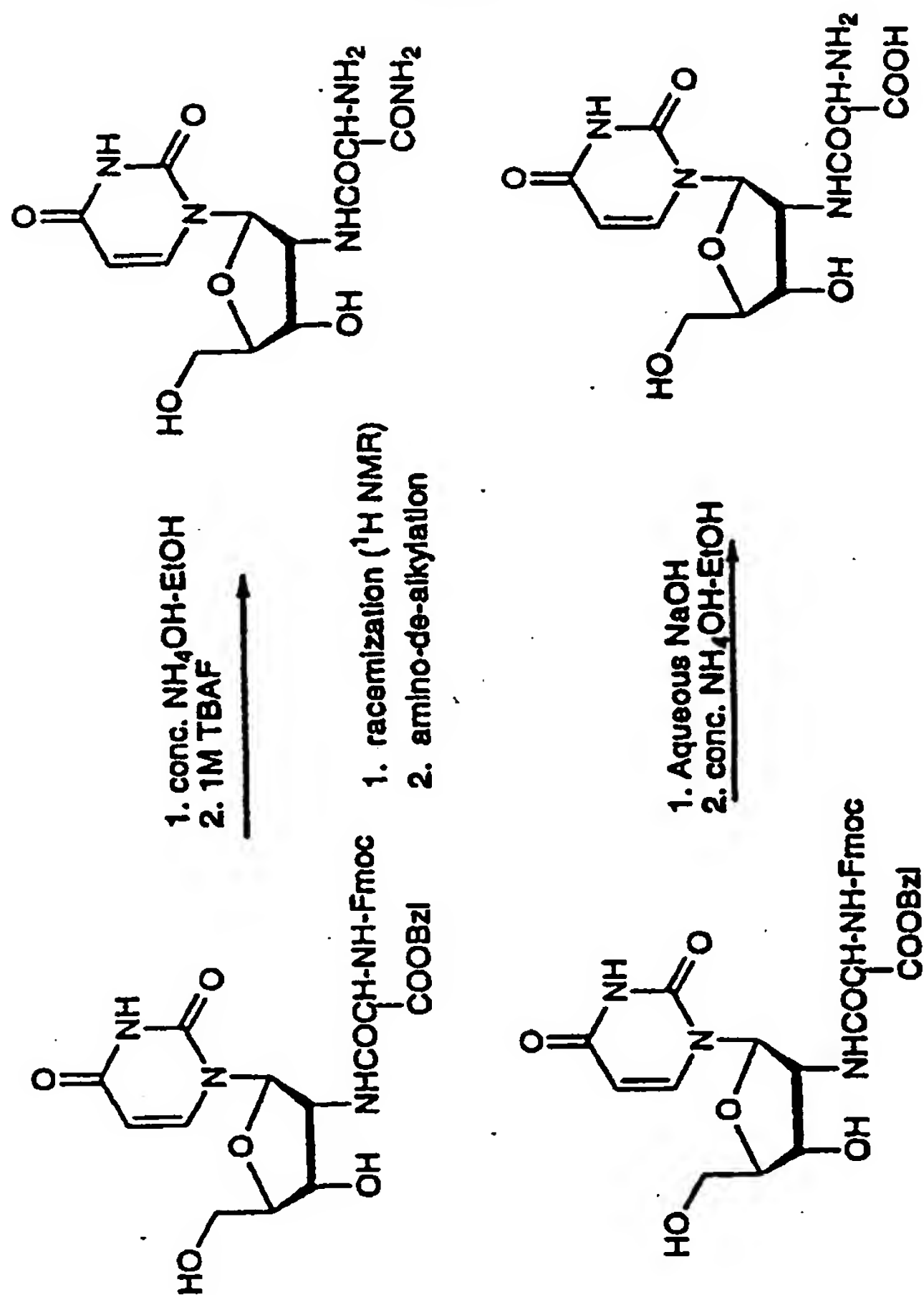


FIG. 93b.

SUBSTITUTE SHEET (RULE 26)

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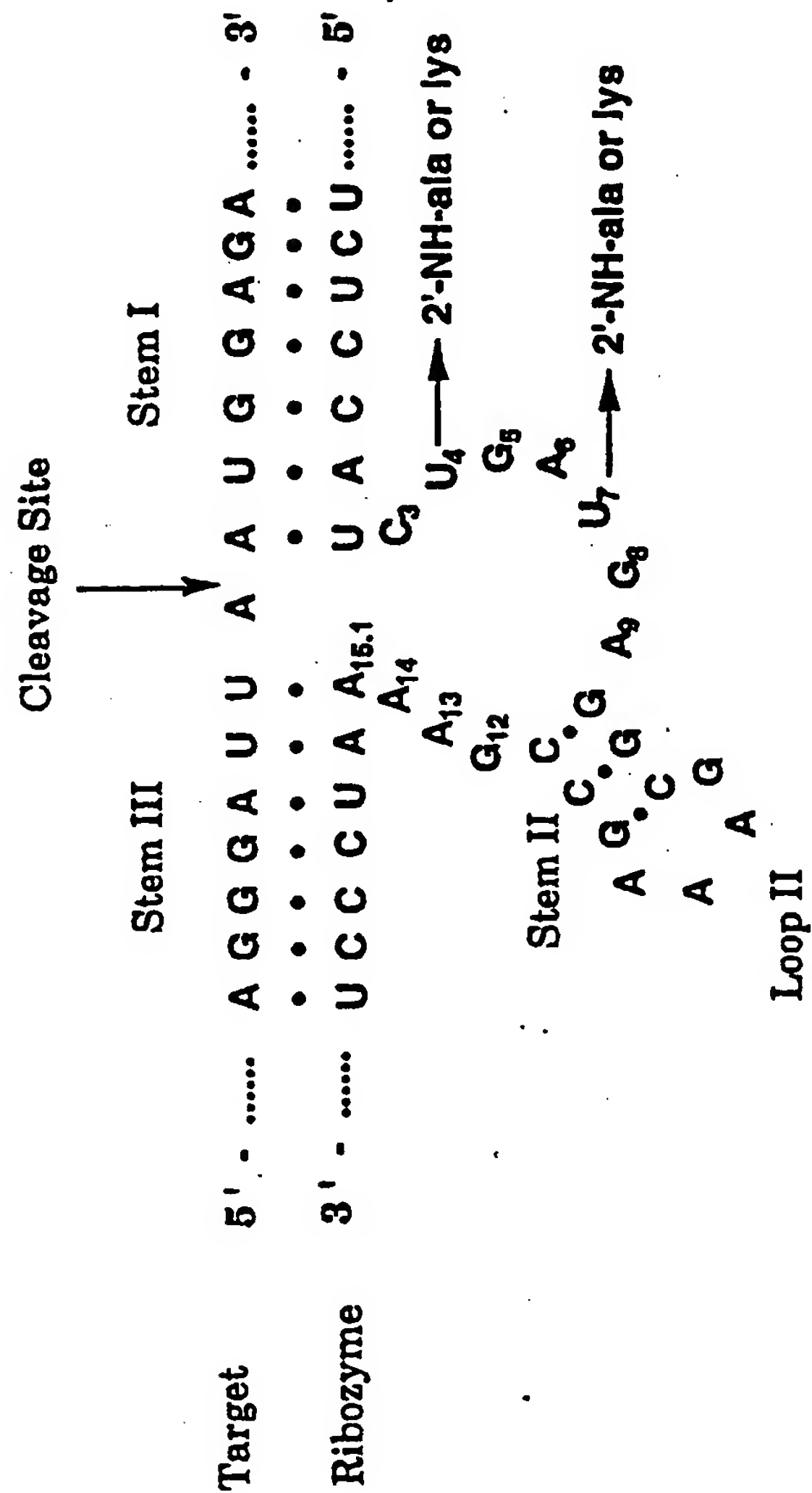
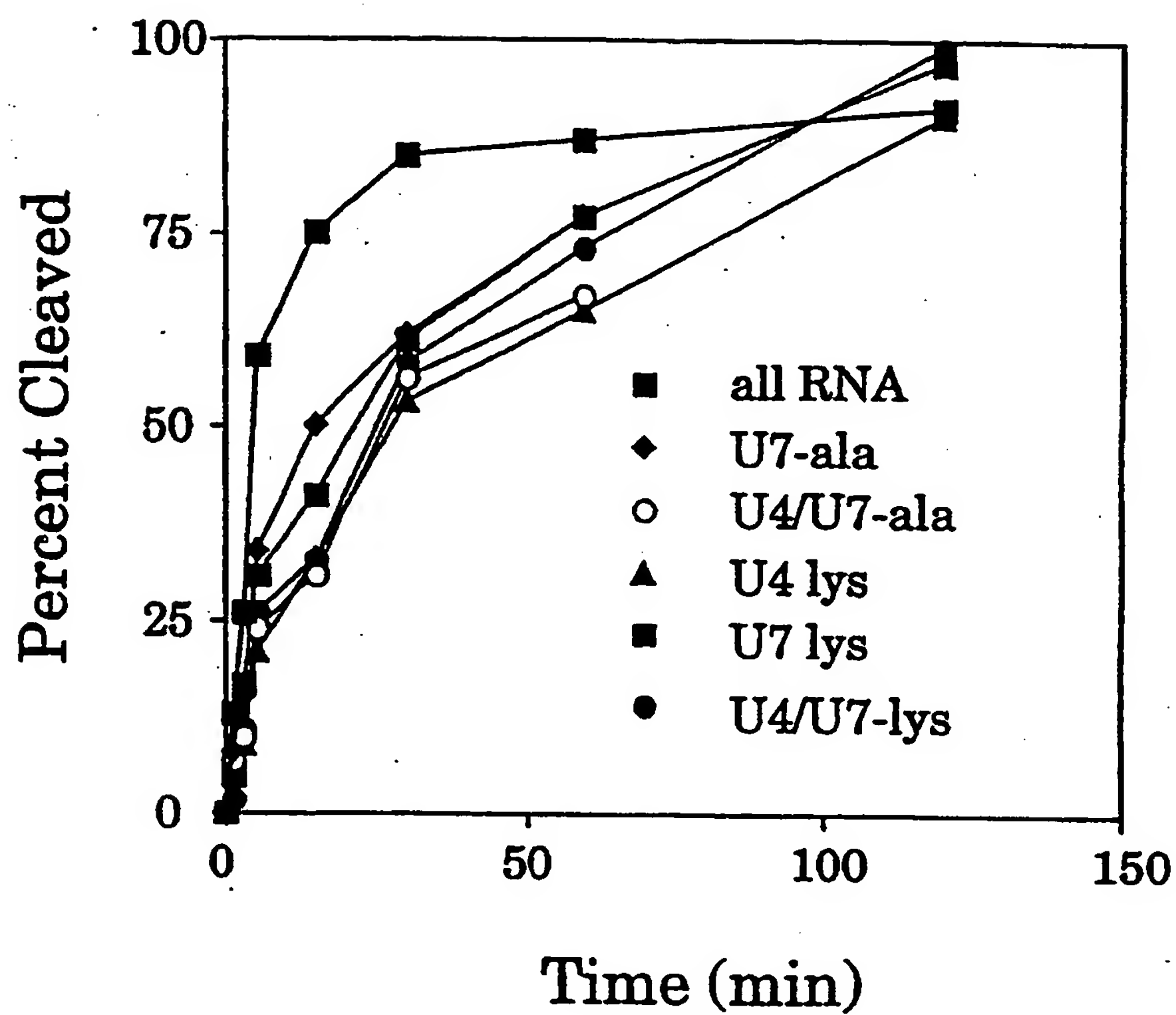


FIG. 94.

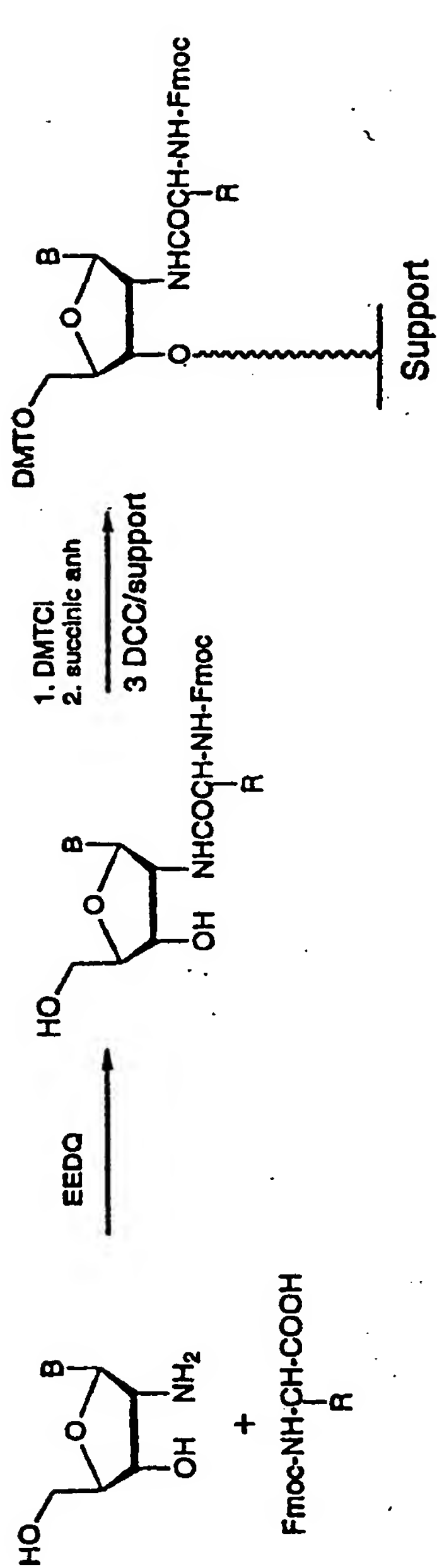
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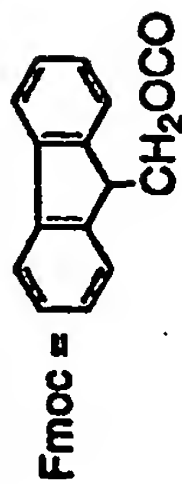
[Ribozyme] = 40 nM [Substrate] = ~1nM

FIG. 95.

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EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline



R = CH₃, CH₂-, (CH₂)₄NH-Fmoc, (CH₂)₄NH-CBZ, CH₂COOBzl
(ala) (phe) (lys) (asp)

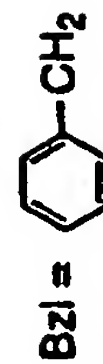
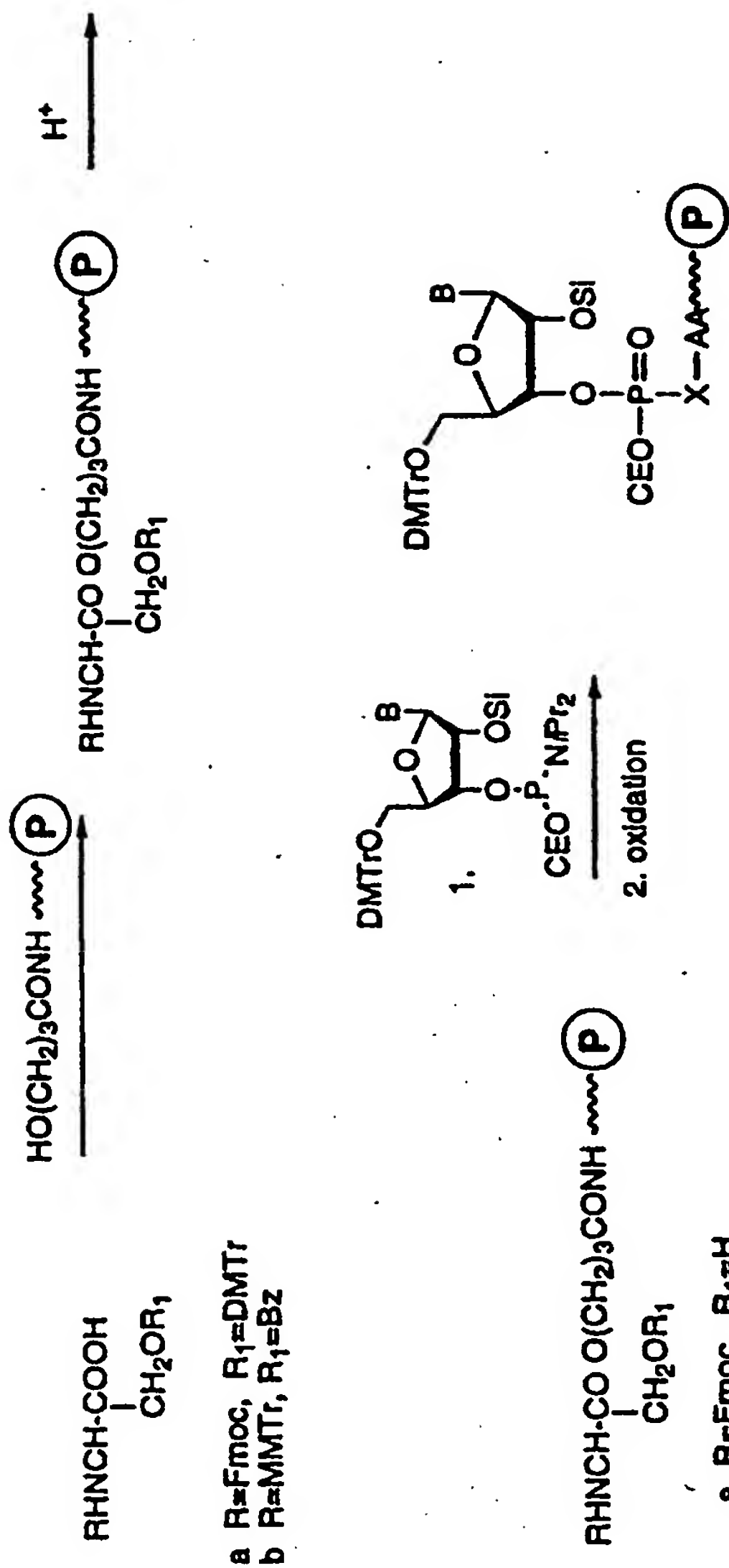


FIG. 96.

SUBSTITUTE SHEET (RULE 26)

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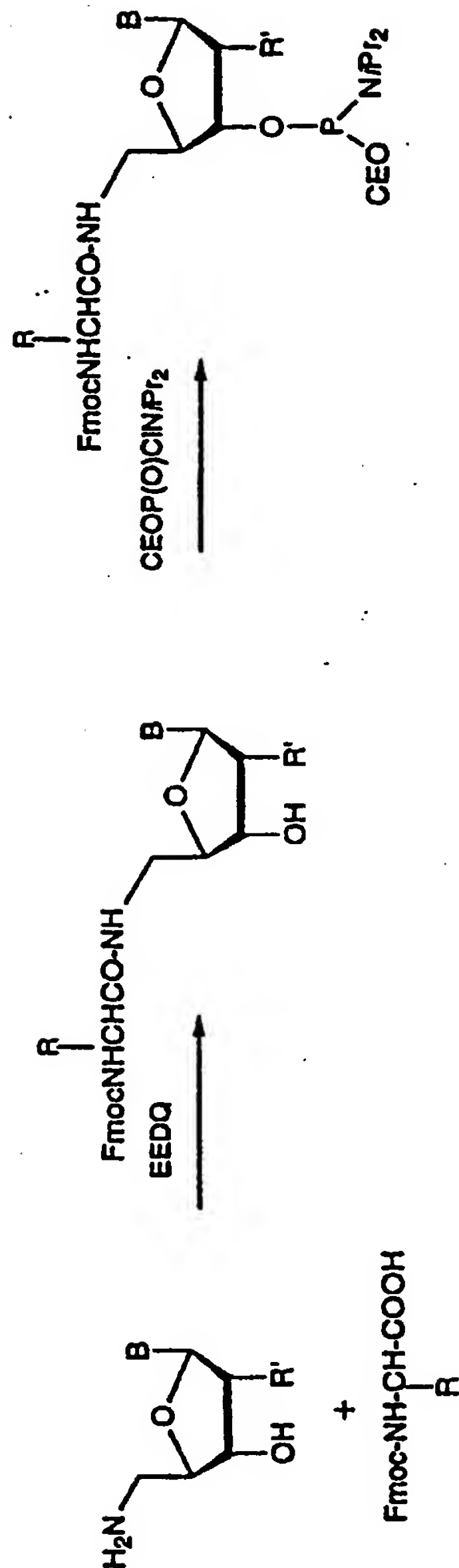
a X=O, AA=CH₂CH(NHFmoc)CO
 b X=NH, AA=CH(CH₂OBz)CO

B= Ura, Cyt^{bz}, Ade^{bz}, Gua^{ibu}, mod. base, H

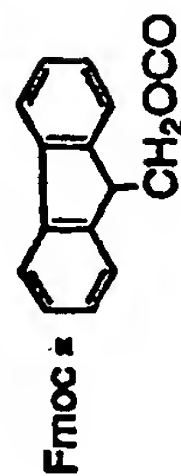
FIG. 97.

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EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline



R = CH₃, CH₂-(c1ccc(cc1)C(=O)OCC) (ala), (CH₂)₄NH-Fmoc, (CH₂)₄NH-CBZ, CH₂COOBzl (lys), (asp)



R' = H, OMe, OTBDMSI

B = Ura, Cyt^{bz}, Ade^{bz}, Gua^{ibu}, mod. base, H

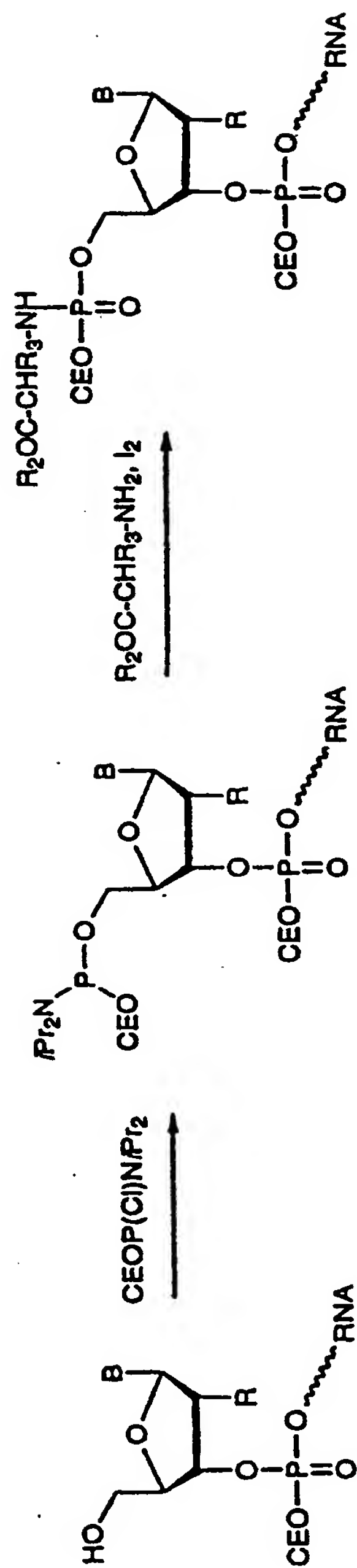
FIG. 98.

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FIG. 99.



B = Ura, Cyt^{bz}, Ade^{bz}, Gua^{ibu}, mod. base, H
 R = H, OCH₃, OTBDMS, Hal, NHR₁
 R₂ = OBzl, peptidyl

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FIG. 100.

Reversion of mutant RNA

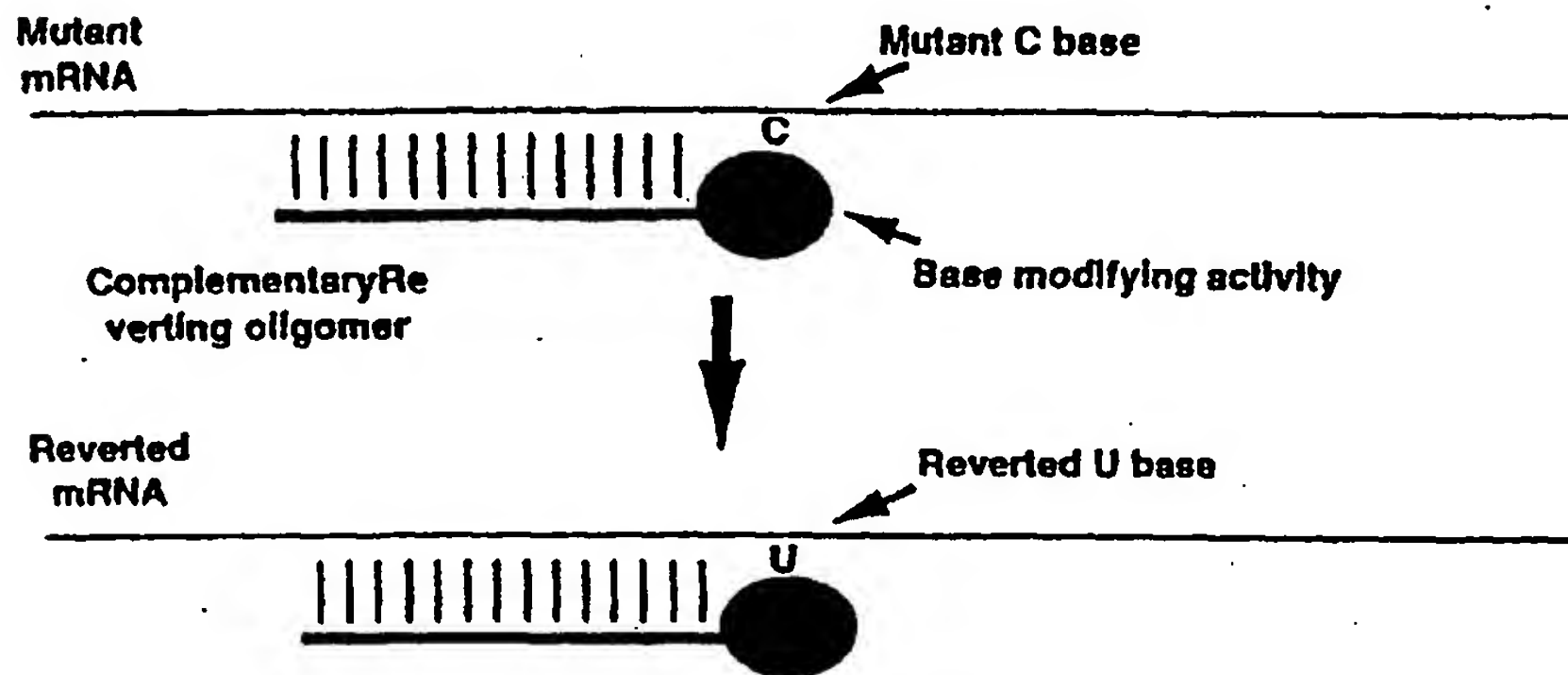
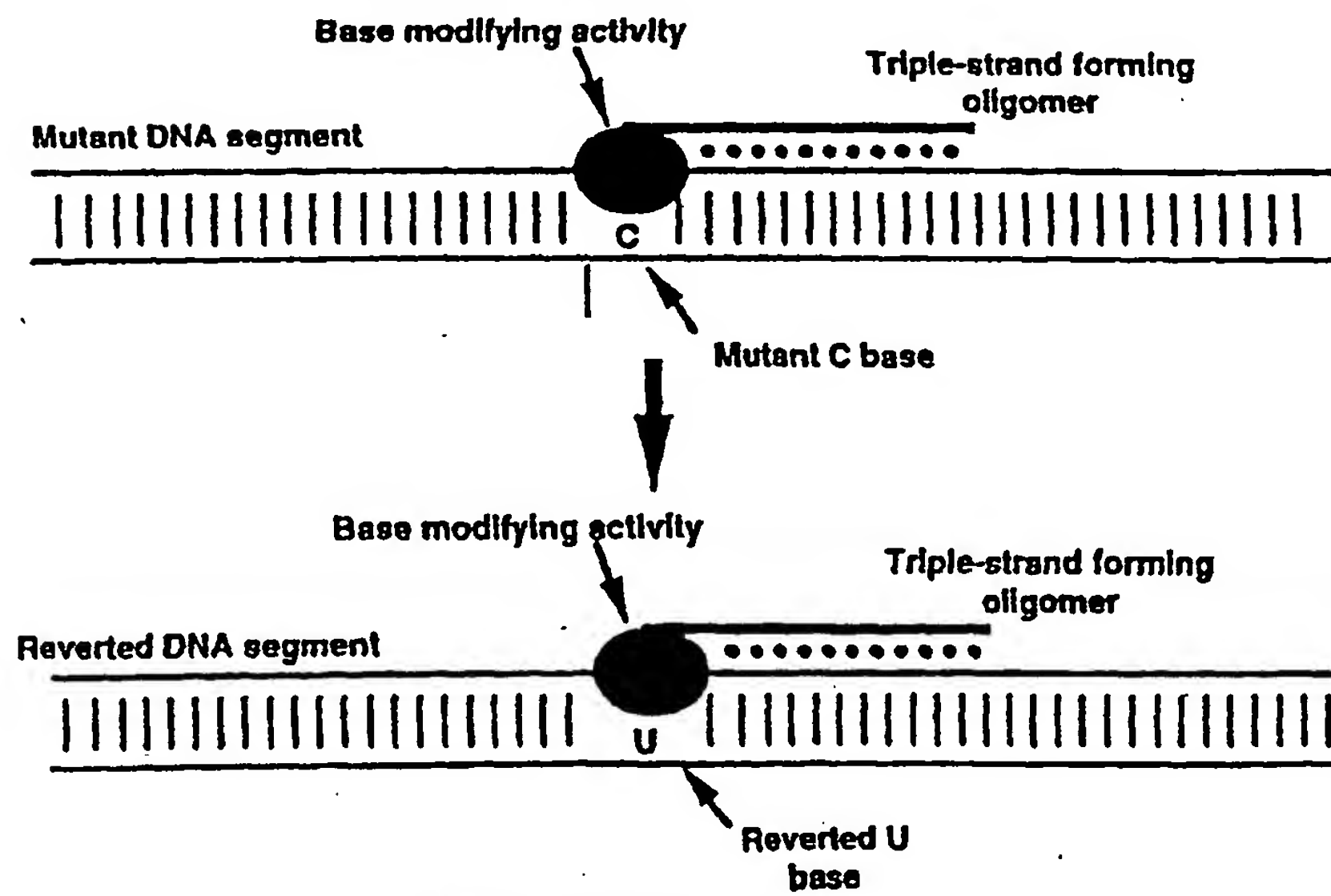


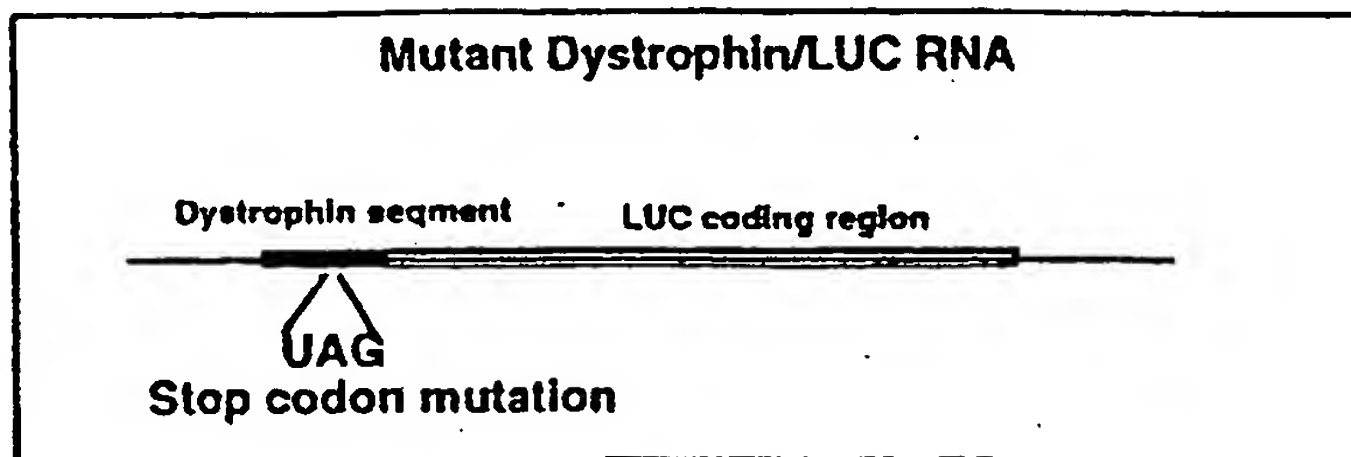
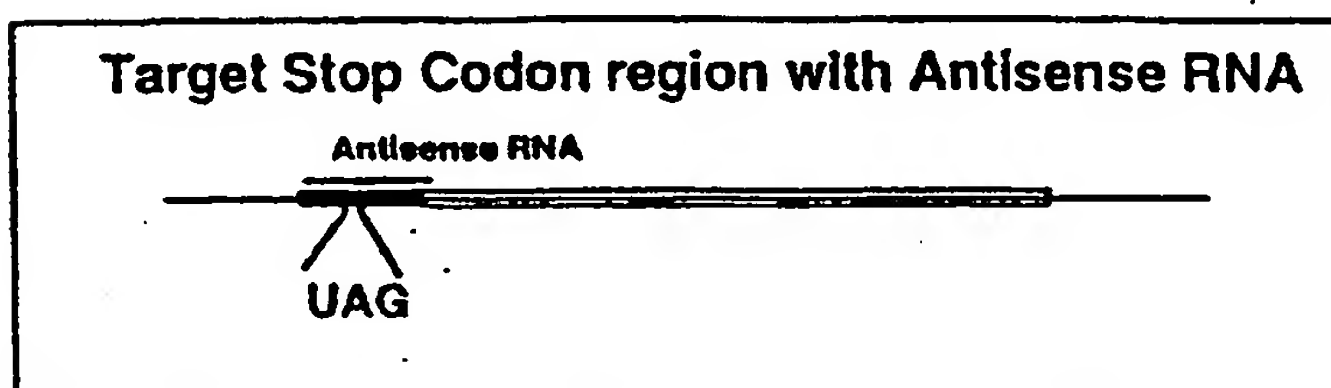
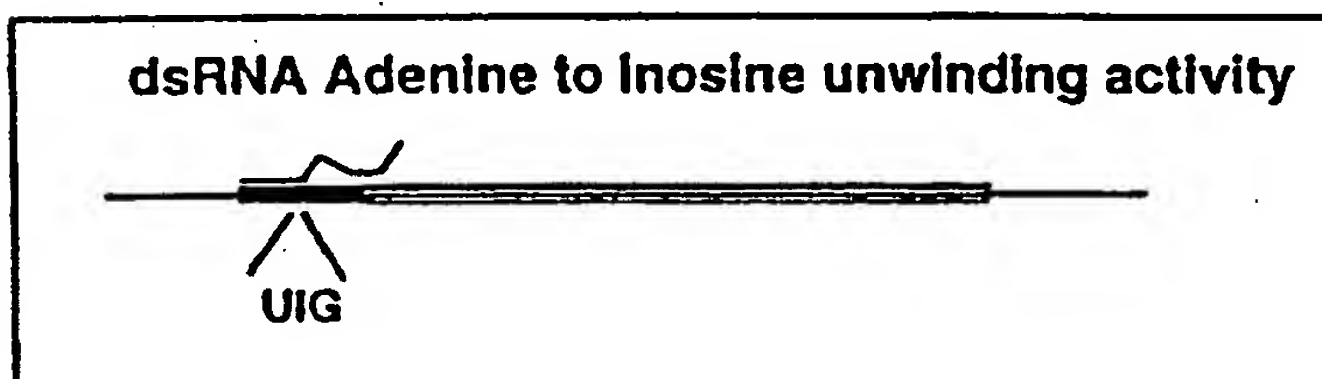
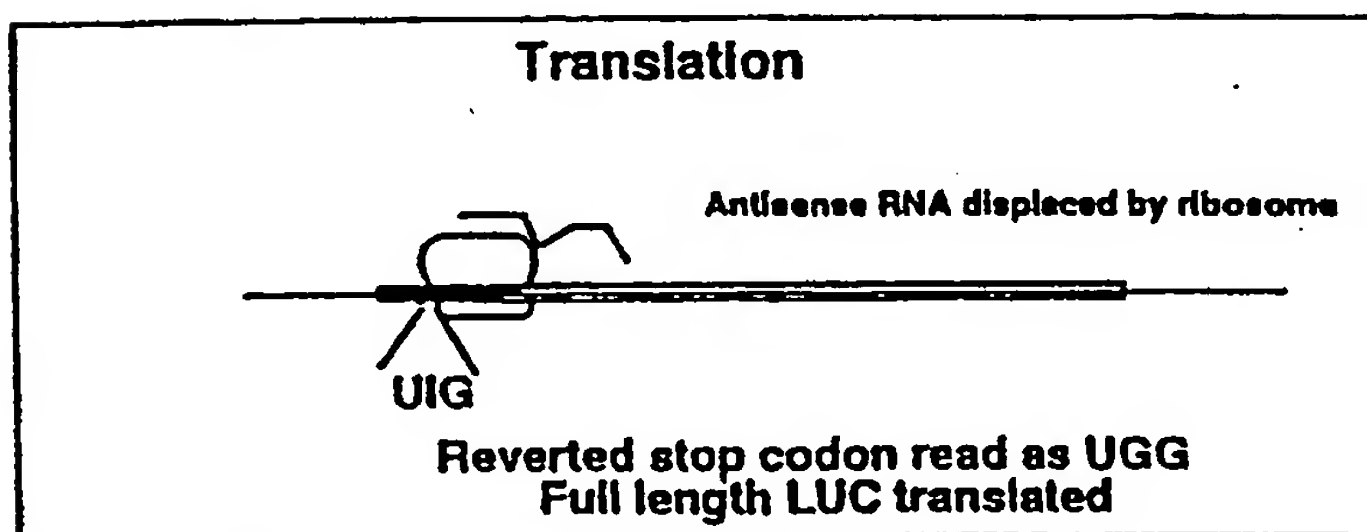
FIG. 101.

Reversion of mutant DNA



SUBSTITUTE SHEET (RULE 26)

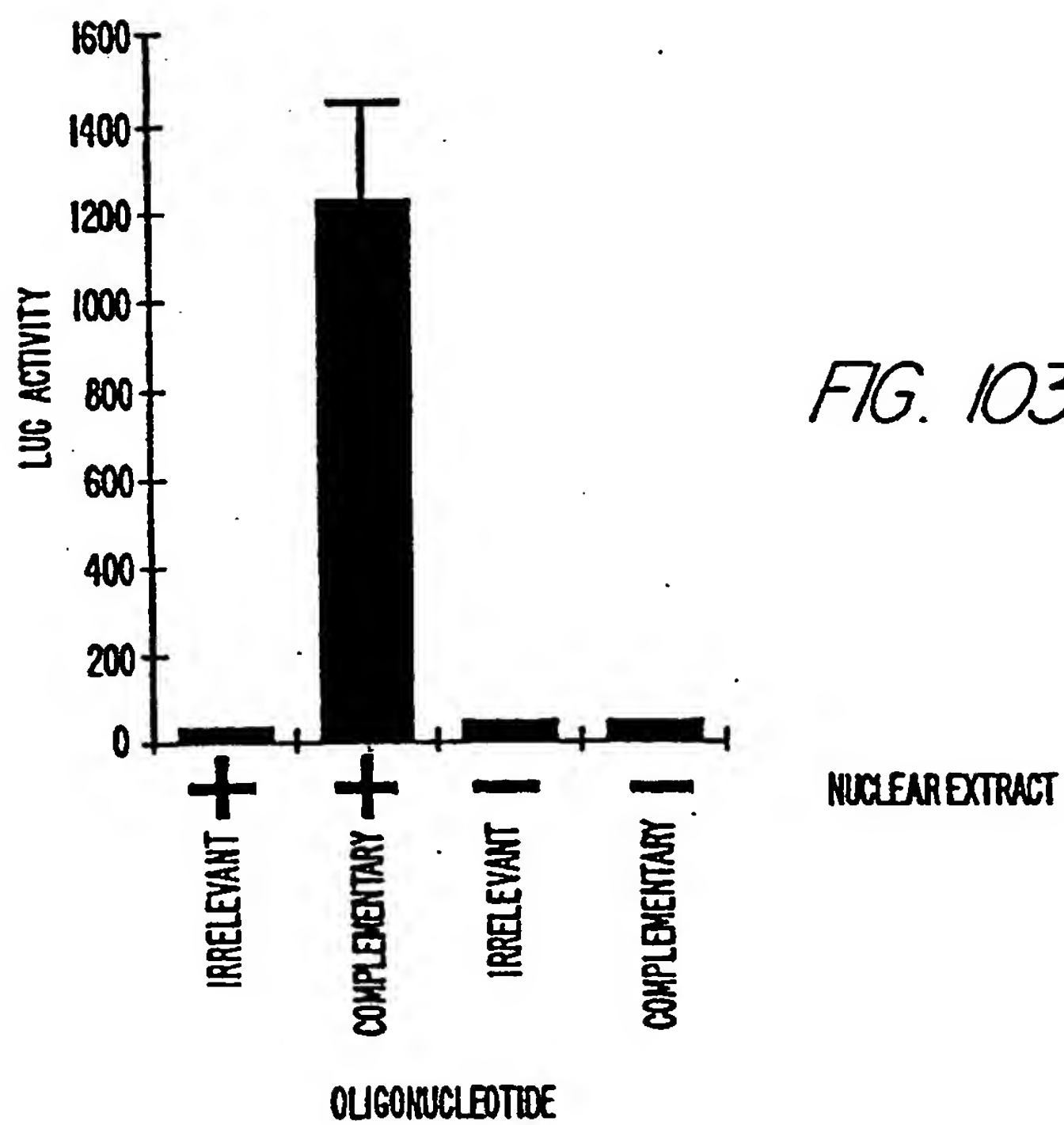
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*FIG. 102a.**FIG. 102b.**FIG. 102c.**FIG. 102d.*

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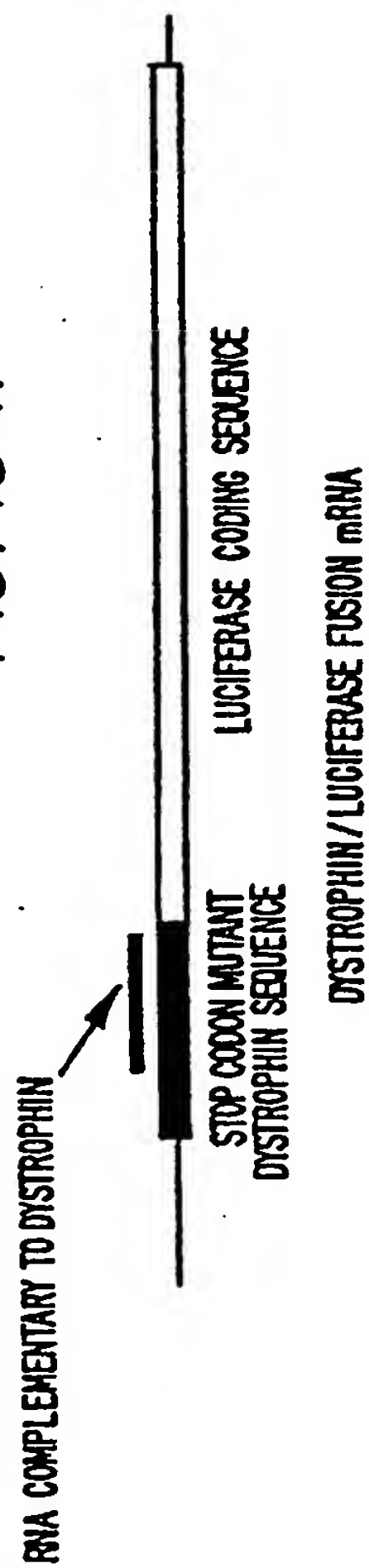


SUBSTITUTE SHEET (RULE 26)

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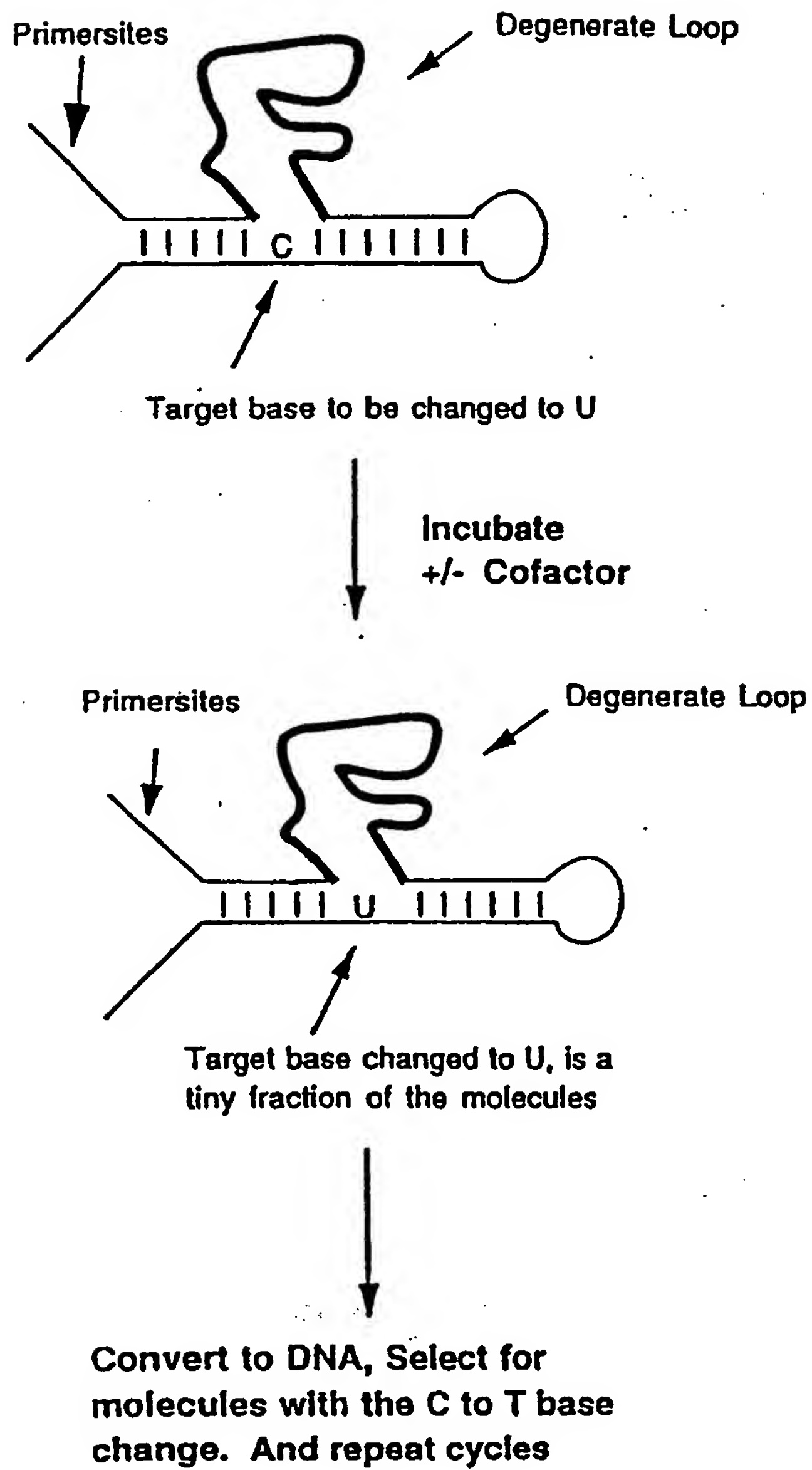
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FIG. 104.



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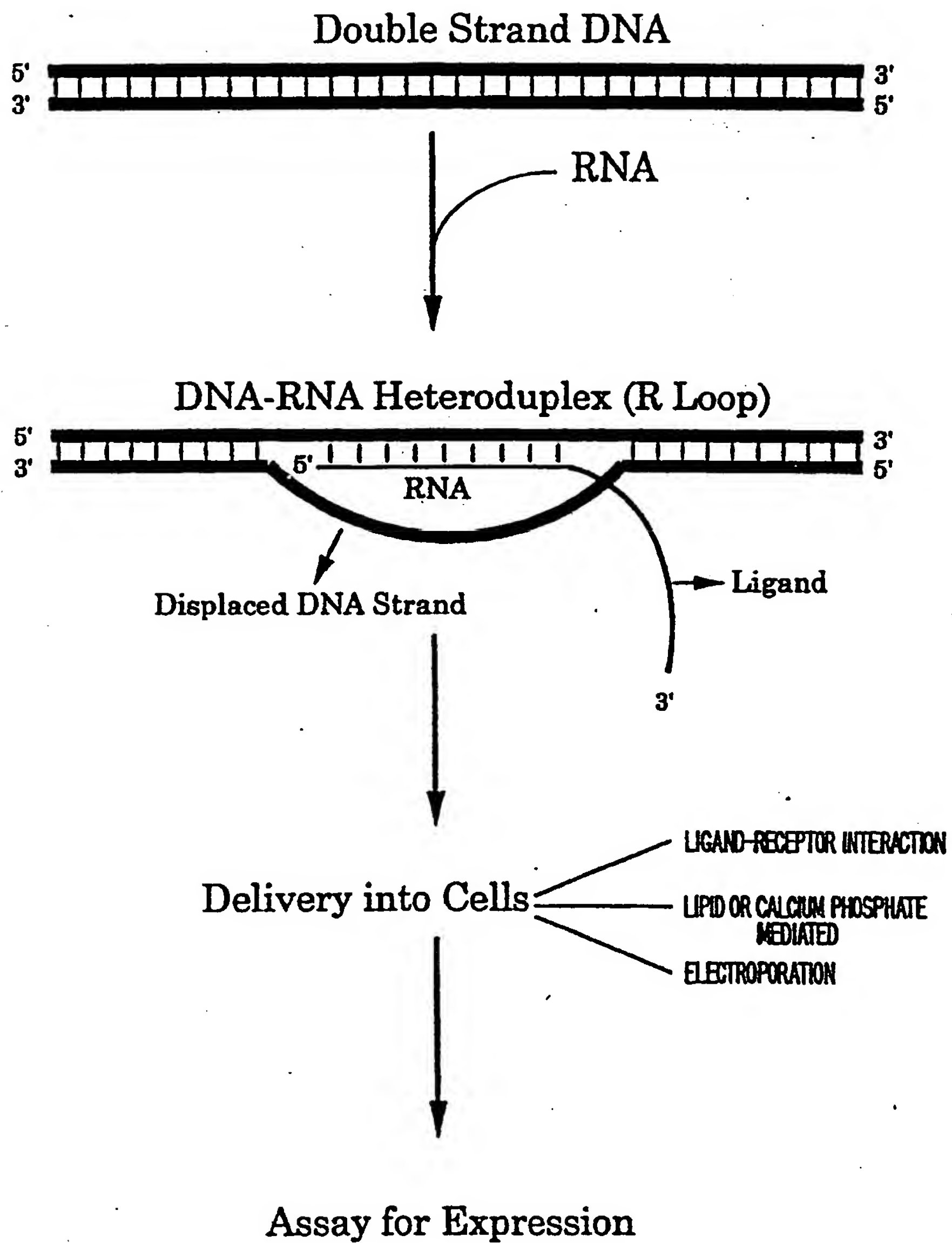
FIG. 105.



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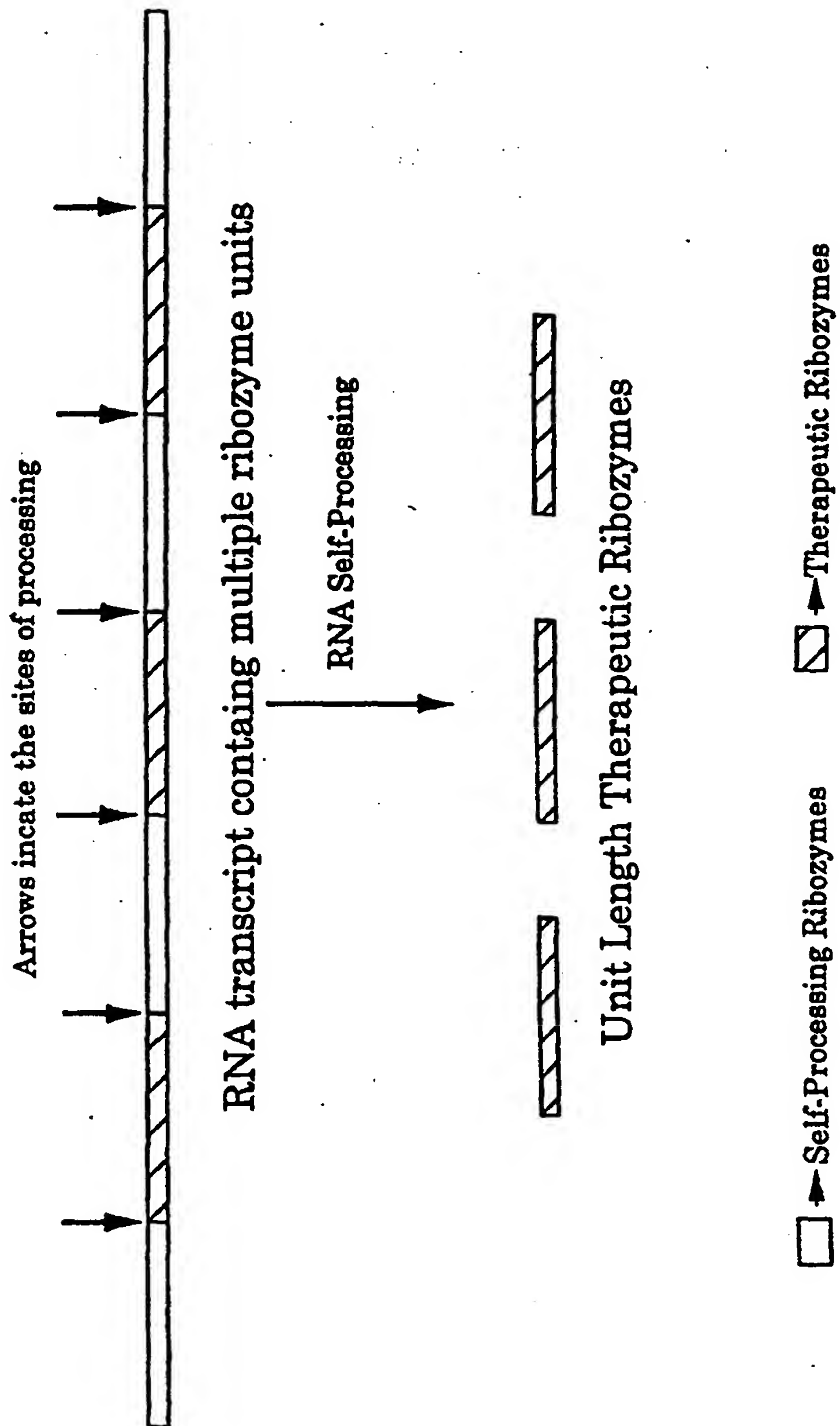
*FIG. 106.*

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FIG. 107



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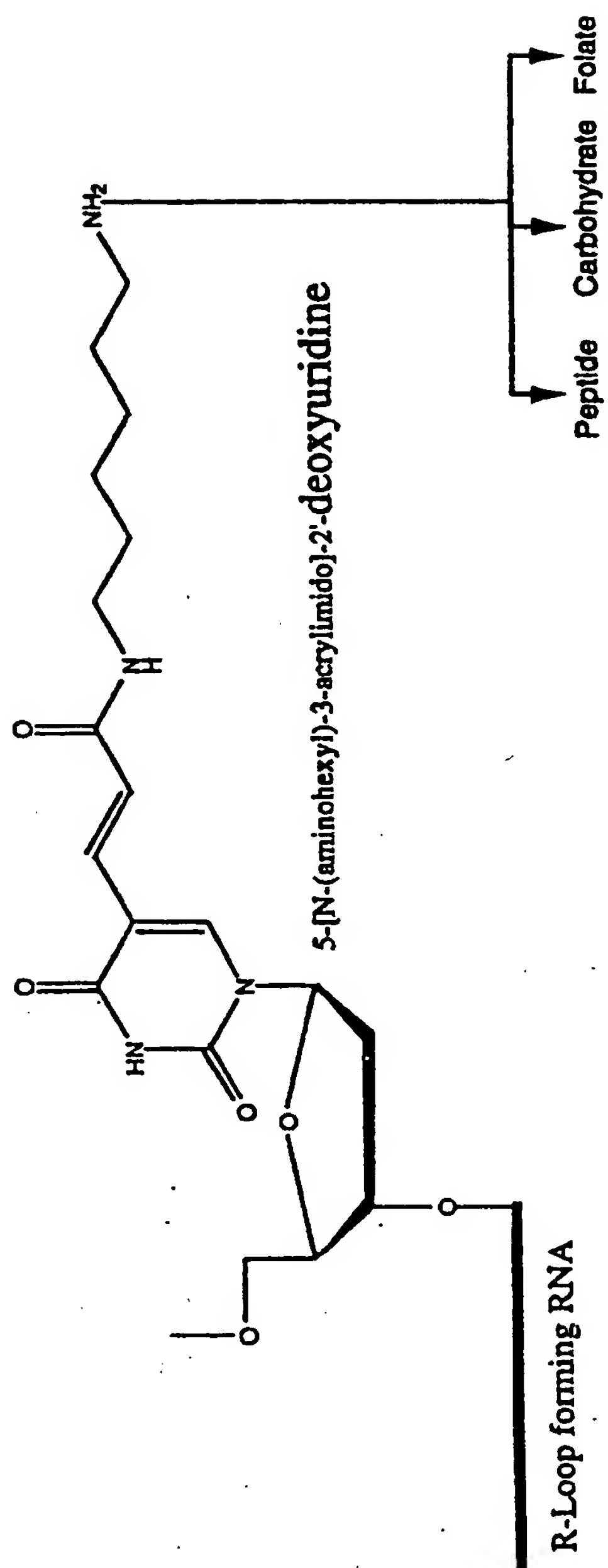


FIG. 108.

SUBSTITUTE SHEET (RULE 26)